AD-A146 242 MICROWAVE BLOOD THAWING: BIOCHEMICAL ANALYSIS OF SMALL SAMPLES OF THAWED RED BLOOD CELLS(U) NAVAL OCEAN SYSTEMS CENTER SAN DIEGO CA N L CAMPBELL JAN 84 1/1

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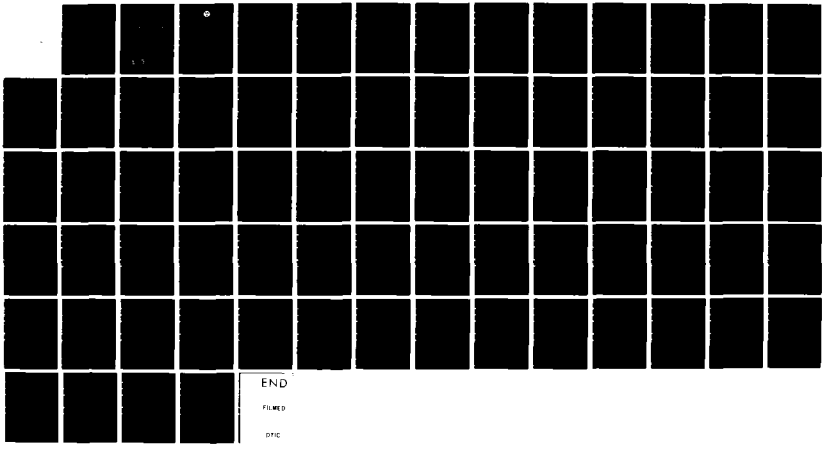

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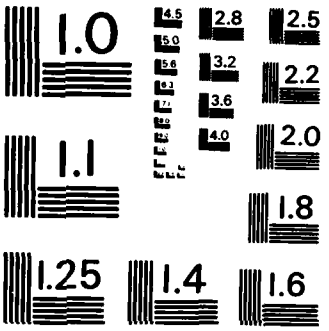

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NOSC TR 970

AD-A146 242

NOSC TR 970

Technical Report 970

MICROWAVE BLOOD THAWING: BIOCHEMICAL ANALYSIS OF SMALL SAMPLES OF THAWED RED BLOOD CELLS

N. L. Campbell

January 1984
January 1979 — July 1982

Prepared for
Naval Medical Research
and Development Command

Approved for public release; distribution unlimited.

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ADMINISTRATIVE INFORMATION

This task was performed for the Naval Medical Research and Development Command, Code 45, Bethesda, MD 20014, under program element 63706N, subproject M0095 (NOSC 514-FA24). The work was carried out by the Naval Ocean Systems Center (NOSC Code 5143), San Diego, CA 92152.

Released by
L.W. Bivens, Head
Biological Sciences Division

Under authority of
H.O. Porter, Head
Biosciences Department

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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NOSC TR 970	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Ocean Systems Center 6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State and ZIP Code) Code 5143 San Diego, CA 92152	7b. ADDRESS (City, State and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research & Development Command 8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State and ZIP Code) Code 45 Bethesda, MD 20014	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 63706N; PROJECT NO: M0095; TASK NO:; WORK UNIT NO: 514-FA24

11. TITLE (Include Security Classification)

MICROWAVE BLOOD THAWING: BIOCHEMICAL ANALYSIS OF SMALL SAMPLES OF THAWED RED BLOOD CELLS

12. PERSONAL AUTHOR(S)
N. L. Campbell

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM Jan 1979 TO Jul 1982	January 1984	71

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Microwave thawing, blood thawing, RBC metabolism, microwave effects, RBC (red blood cell), microwave exposure

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Frozen storage of packed red blood cells (pRBCs) increases the 35 day shelf life of liquid storage to several years, thereby decreasing the wastage of unused blood and increasing the availability of rare blood types. These frozen pRBC units are currently thawed in a warm water bath, a practice which has been reported to contaminate a small percentage of units. A dry thawing method would eliminate the contamination risk encountered when using water baths. One potential dry heat source is microwave energy. However, before microwave thawing can be used clinically, its effects upon the structure and function of red blood cells (RBCs) must be thoroughly examined. The goal of this study is to determine the feasibility of using microwave energy as a dry thaw method for frozen pRBCs.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified	Agency Accession DN787 507
22a. NAME OF RESPONSIBLE INDIVIDUAL N. L. Campbell	22b. TELEPHONE (Include Area Code) (619) 225-6542	22c. OFFICE SYMBOL Code 5210

DD FORM 1473, 84 JAN

83 APR EDITION MAY BE USED UNTIL EXHAUSTED
ALL OTHER EDITIONS ARE OBSOLETE

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SECURITY CLASSIFICATION OF THIS PAGE

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LIST OF ABBREVIATIONS

ACD	Acid citrate dextrose
ATP	Adenosine-5'-triphosphate
CPD	Citrate phosphate dextrose
DPG	2,3-diphosphoglycerate
FDA	Food and Drug Administration
gRBC	Glycerolized red blood cell
%H	Percent hemolysis
GSH	Glutathione, reduced
Hb	Hemoglobin
Hb_c	Cellular hemoglobin
Hb_c%	Cellular hemoglobin in g/100 ml solution (g%)
Hb_s	Supernatant hemoglobin
Hb_s%	Supernatant hemoglobin in g/100 ml supernatant (g%)
Hb_t	Total hemoglobin
Hb_t%	Total hemoglobin in g/100 ml solution (g%)
Hct	Hematocrit
MCH	Mean cellular hemoglobin
MCHC	Mean cellular hemoglobin concentration
MCV	Mean cellular volume
metHb	Methemoglobin
MT	Microwave thaw
MT-RBC	Microwave thawed red blood cell
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide, reduced
pRBC	Packed red blood cell
%R	Percent recovery
RBC	Red blood cell
RBC#	Red blood cell count
sn	Solution
sp	Supernatant
thRBC	Thawed red blood cell
V/V	Volume to volume

LIST OF ABBREVIATIONS (Continued)

WBC	White blood cell
whRBC	Whole blood
wRBC	Washed red blood cell
WT	Water bath thaw
WT-RBC	Water bath thawed red blood cell
W/V	Weight to volume

INTRODUCTION

PROBLEM

Frozen storage of packed red blood cells (pRBCs) increases the 35 day shelf life of liquid storage to several years, thereby decreasing the wastage of unused blood and increasing the availability of rare blood types. These frozen pRBC units are currently thawed in a warm water bath, a practice which has been reported to contaminate a small percentage of units (U.S. Dept. of Health, Education and Welfare, 1976b; Szymanski and Carrington, 1977; U.S. Dept. of Health, Education and Welfare, 1979). A dry thawing method would eliminate the contamination risk encountered when using water baths.

One potential dry heat source is microwave energy. However, before microwave thawing can be used clinically, its effects upon the structure and function of red blood cells (RBCs) must be thoroughly examined. The goal of this study is to determine the feasibility of using microwave energy as a dry thaw method for frozen pRBCs.

BACKGROUND

Blood banking, the collection and storage of blood for later use, provides quick access to a treatment for persons suffering from a loss of blood or a blood-related disease. The history of blood banking reveals a continuing quest for methods to increase the shelf life of stored blood. The first step toward this goal was the discovery of an anticoagulant to prevent blood clotting before transfusion. In 1914, Mustin used citrate to prevent coagulation of drawn blood which was transfused within minutes into a patient. Rous and Turner, in 1916, discovered the value of adding glucose as an energy source in extending the life span of stored whole blood. Acidification of the citrate dextrose mixture was shown by Maizels in 1940 to double the shelf life of stored whole blood (Hurn, 1968). Currently, the addition of various nucleosides to the anticoagulant has been shown to greatly preserve RBC metabolism, and increase the shelf life of RBCs stored at 4°C (Simmons, 1968; Valeri, 1974a; 1976; Valeri et al., 1977; 1979).

A unit of whole blood can be separated into various therapeutic components including pRBCs, white blood cells (WBCs), plasma, platelets, and plasma proteins (albumin, gamma globulin, clotting factors, and fibrinogen) (Rapaport, 1971). The individual components are either transfused immediately, stored in a liquid state, or stored in a frozen state. The use of blood components maximizes the benefits obtained from a single unit of blood and decreases the risk of transfusion reactions (adverse reactions by the patient to the transfused component, usually because of blood group incompatibilities) (Valeri, 1975). Transfusion of blood components has largely replaced transfusion of whole blood in modern hospitals. For example, a common need in transfusion therapy is to enhance the blood's oxygen-carrying ability. Packed RBCs are often used for the treatment of patients with chronic anemia and congestive heart failure, and for surgical patients both before and after surgery (Williams, 1972).

Frozen storage, first attempted by Luyet in 1949 (Turner, 1970), increased the shelf life of blood components from 21 days in a liquid state at 4°C to 3 years, frozen at -65°C (American Association of Blood Banks, 1977). The main advantages of freezing blood for storage are as follows:

1. All blood groups and types, including rare types, become more available;

2. Autotransfusion (transfusion of the patient's own blood, drawn earlier and stored) decreases the chance of transfusion reactions;

3. Patients undergoing organ transplantation have a decreased risk of tissue rejection because of the few WBCs associated with frozen-stored RBCs;

4. Wastage due to the outdating of liquid-stored blood is less;

5. Component therapy is enhanced by the purity of the frozen RBC, plasma, WBC, or plasma protein units; and

6. Transfer of serum hepatitis is decreased (NRC, Div. of Medical Sciences, 1973).

The military community especially benefits from the use of frozen blood in combat since:

1. Combat-zone blood banks often receive blood 10-12 days post-collection;

2. Demands for blood are sporadic, creating excessive outdating of liquid-stored blood accompanied by occassional severe shortages;

3. Rare types, for example Rh-negative, are limited in the field; and

4. Donations in the field are difficult to obtain, and donors are often unsatisfactory (Moss et al., 1968).

Problems associated with freezing, such as (1) cellular dehydration and mechanical damage to the membrane due to ice crystal formation, and (2) protein denaturation from high salt concentrations, are encountered in the slow freezing of cellular blood components (Luyet, 1965; Mazur, 1965; Rapatz and Luyet, 1973). Slow freezing, placing the cellular component in a mechanical freezer at -80°C, creates large ice crystals extracellularly and high concentrations of solutes intracellularly, denaturing the proteins.

Fast freezing, immersion of the blood in liquid nitrogen (-197°C) for five minutes and storage in gas phase liquid nitrogen at -150°C, eliminates many of the problems associated with slow freezing. The fast freeze allows only small ice crystals to form, minimizing the potential for mechanical damage. And, by quickly freezing the intracellular proteins into a physically inert state, denaturation is prevented in the hostile environment of high salt concentrations. However, the fast freezing method has limitations which make it undesirable for routine use:

1. The specimens must be frozen in thin layers to increase the freezing rate of all cells;

2. The frozen specimens must be thawed very rapidly; and

3. Liquid nitrogen storage is expensive.

Cryoprotective agents, such as glycerol, have been routinely added during slow freezing to minimize the formation of ice crystals and the denaturation of proteins. Unfortunately, intracellular glycerol can cause cells to swell and burst when placed in vivo. The glycerol, therefore, must be washed out of the cells before transfusion.

Thawing the frozen blood components presents problems similar to those encountered in freezing. Slow thawing allows recrystallization of small ice crystals into larger, damaging ice crystals. Also, proteins are exposed longer to high solute concentrations while they are in a physically pliable (denaturable) state. The cryoprotectant aids in decreasing detrimental effects of slow thawing. Fast thawing eliminates many of the adverse effects of slow thawing, but nonuniform heating can cause hot spots where portions of the sample can be overheated and destroyed.

Currently, blood banks use glycerol as a cryoprotectant and use slow freeze, slow thaw methods. Approximately 450 ml of donated whole blood are added to 63 ml of anticoagulant, citrate phosphate dextrose adenine (CPDA-1). The whole blood is centrifuged and most of the supernatant plasma is removed, leaving pRBCs with hematocrit between 60 and 80%, and a volume between 250 and 300 ml.

Packed cells to be frozen must be stored less than six days at 4°C. These cells are prepared for freezing by adding approximately 300 to 400 ml of 57% (grams per 100 ml solution) glycerol. The glycerolized RBCs (gRBCs) are transferred to a 3-liter plastic bag which is placed in a metal or cardboard container and is then placed in a mechanical freezer between -65°C and -80°C. The current FDA regulations allow a maximum of three years storage of pRBCs in the frozen state, though storage for over 10 years has been shown to be successful (AABB, 1977). When a unit is needed for transfusion, it is removed from the freezer and placed in a warm water bath (37°C) until it is thawed (10 to 20 minutes). The glycerol must then be washed out of the thawed blood. Washing is automated, using serial centrifugation of the cells with varying concentrations of NaCl solutions. Following wash, the cells are resuspended in a solution of 0.9% NaCl with 0.2% dextrose.

In the recent past, bacterial contamination of a unit of blood has sometimes resulted in serious, even fatal, transfusion reactions. Contamination was usually caused by frequent entering and sampling of the unit, by bacteremic donors, or by improper phlebotomy techniques (U.S. Dept. of Health, Education and Welfare, 1976c). In 1951, the Bureau of Biologics (FDA) imposed the current "24 hour rule," which specifies that once the unit of blood has been entered, the blood must be transfused or discarded within 24 hours. Within this 24 hour period, the unit of blood is considered safe for transfusion.

The 24 hour rule has met with controversy since it severely limits the use of frozen blood and causes much wastage of thawed, unused units (U.S. Dept. of Health, Education and Welfare, 1976c; 19771; 1977b; 1978; U.S. Dept. of Health, Education and Welfare, 1976b; Valeri et al., 1977; 1979; Valeri, 1976). Techniques are now available for maintaining sterile entry sites, thus greatly reducing contamination during glycerolizing and deglycerolizing procedures (U.S. Dept. of Health, Education and Welfare, 1976d; U.S. Dept. of Health, Education and Welfare, 1976a). Extending this 24 hour post-thaw storage period to at least 72 hours would significantly reduce wastage of this expensive product.

A major source of contamination of frozen stored blood is the 37°C water bath used to thaw the units as exemplified by the following three studies. First, the U.S. Dept. of Health, Education and Welfare (1976b) studied five blood processing laboratories and found a 2.4% incidence of contamination following thaw and wash of red blood cells. In one laboratory, 6.9% of the units were contamined, the majority with Mima polymorpha var. oxidans. This strain of bacteria was also found in the water bath used for thawing, and identified the bath as a major contamination hazard. Following the use of a sterilizing agent in the bath, 1.1% of the units were still contaminated. In the second study, Szymanski and Carrington (1977) claimed a 5% contamination incidence in thawed washed red blood cells. Following the daily addition of an antiseptic to the water of the thawing bath, contamination still remained at 1%. Finally, the Department of Health, Education, and Welfare (1979) reported that in January and February of 1979, three patients in a large university hospital acquired serious infection by the bacterium Pseudomonas cepacia following the receipt of cryoprecipitates. The water bath used to thaw these cryoprecipitates contained approximately 2×10^8 per ml of the same bacterial strain, even though the bath was cleaned with povodine-iodine and fresh water was added every day. Given this unavoidable contamination from water baths in civilian hospitals, the contamination problem of water baths in the field environment of military hospitals could be even more serious and should be eliminated.

POTENTIAL ADVANTAGES AND DISADVANTAGES OF MICROWAVE THAWING

Microwave energy offers a dry-thawing method that would eliminate the contamination introduced by water baths. Microwaves fall within the electromagnetic frequency spectrum between 300 MHz and 300 GHz. This type of non-ionizing radiation generates heat from the friction of dipole molecules (e.g., H_2O) orienting within the field. Biological tissues, because of their high water content, respond well to heating with microwave energy. Because of the large penetration depth, microwave energy has the added advantage of reducing the time required in traditional conduction heating. Microwave thawing has been studied in relation to frozen preserved kidneys (Rajotte et al., 1974) and frozen granulocytes (Georgia Inst. of Tech., 1976) with encouraging results and viable cells post-thaw.

Unfortunately, microwave thawing is not always uniform. The electrical characteristics of a material can vary significantly between its solid and liquid state. Water absorbs microwave energy at a much higher rate than ice.

Therefore, the portions of the frozen sample that thaw first absorb more energy than the still frozen portion, heat faster, and become hot spots. This process has been termed "thermal runaway." Thermal damage can be avoided by uniform heating — raising the temperature of all portions of the sample at the same rate.

The major destructive effect of microwave heating may be denaturation of proteins (Cleary, 1973; U.S. Dept. of Health, Education and Welfare, 1970). As the protein absorbs microwave energy, new molecular bonds can be created or old ones destroyed, thus altering the structure of the molecule. Altering the structure of many of the RBC proteins could adversely affect either their structural or metabolic functions, thereby decreasing the RBC's effectiveness in transfusion therapy.

PURPOSE OF STUDY

The purpose of this study is to determine the in vitro viability of microwave-thawed RBCs (MT-RBCs) by comparing MT-RBCs with water bath-thawed RBCs (WT-RBCs). By measuring in vitro morphologic and metabolic parameters of blood thawed by both methods, potentially harmful effects of microwave thawing can be detected.

MATERIALS AND METHODS

EXPERIMENTAL PROTOCOL

Each unit of pRBC used in this study was glycerolized, divided into approximately 80 aliquots of 3.5 ml each, and frozen at -75°C for at least 24 hours (Figure 1). Samples from the same unit of blood were then periodically removed from the freezer for both water bath thawing (WT) and microwave thawing (MT). The WT- and MT-RBCs were washed side by side using the same wash solutions and similar conditions. Testing of the metabolism of the washed RBCs (wRBCs) continued for 6 hours after washing. Handling of both WT- and MT-RBCs was identical, the only difference in treatment being the method of thaw. Results from WT samples provided a baseline for comparing results from MT samples. Consistent differences in RBC characteristics between WT- and MT-RBCs should reflect any effects due to the thaw method, and indicate potential hazards of the MT process.

PREPARATION OF BLOOD FOR FREEZING

Eight full units (labelled 15, and 17-23 in this report) of pRBCs collected in citrate phosphate dextrose (CPD) were obtained from either the Naval Regional Medical Center (San Diego) Blood Bank, or the San Diego Blood Bank. Six of these units (units 17, 18, and 20-23) were outdated when received (greater than 21 but less than 28 days post-collection), while the other two (units 15 and 19) were fresh (less than 6 days post collection).

To prepare a unit for freezing, the cells were brought to room temperature. A small aliquot was taken for initial testing which included the determination of morphologic and metabolic characteristics. Morphologic measurements included:

- total hemoglobin concentration (Hb_t%),
- supernatant hemoglobin concentration (Hb_s%),
- cellular hemoglobin concentration (Hb_c%),
- hematocrit (Hct),
- red blood cell count (RBC#),
- mean cellular volume (MCV),
- mean cellular hemoglobin concentration (MCHC), and
- mean cellular hemoglobin (MCH).

Metabolic measurements included:

- glucose concentration,
- lactate concentration,
- pyruvate concentration,
- adenosine triphosphate (ATP) concentration,
- 2,3-diphosphoglycerate (DPG) concentration, and
- reduced glutathione (GSH) concentration.

Not all parameters were measured on all units at this step of the preparation.

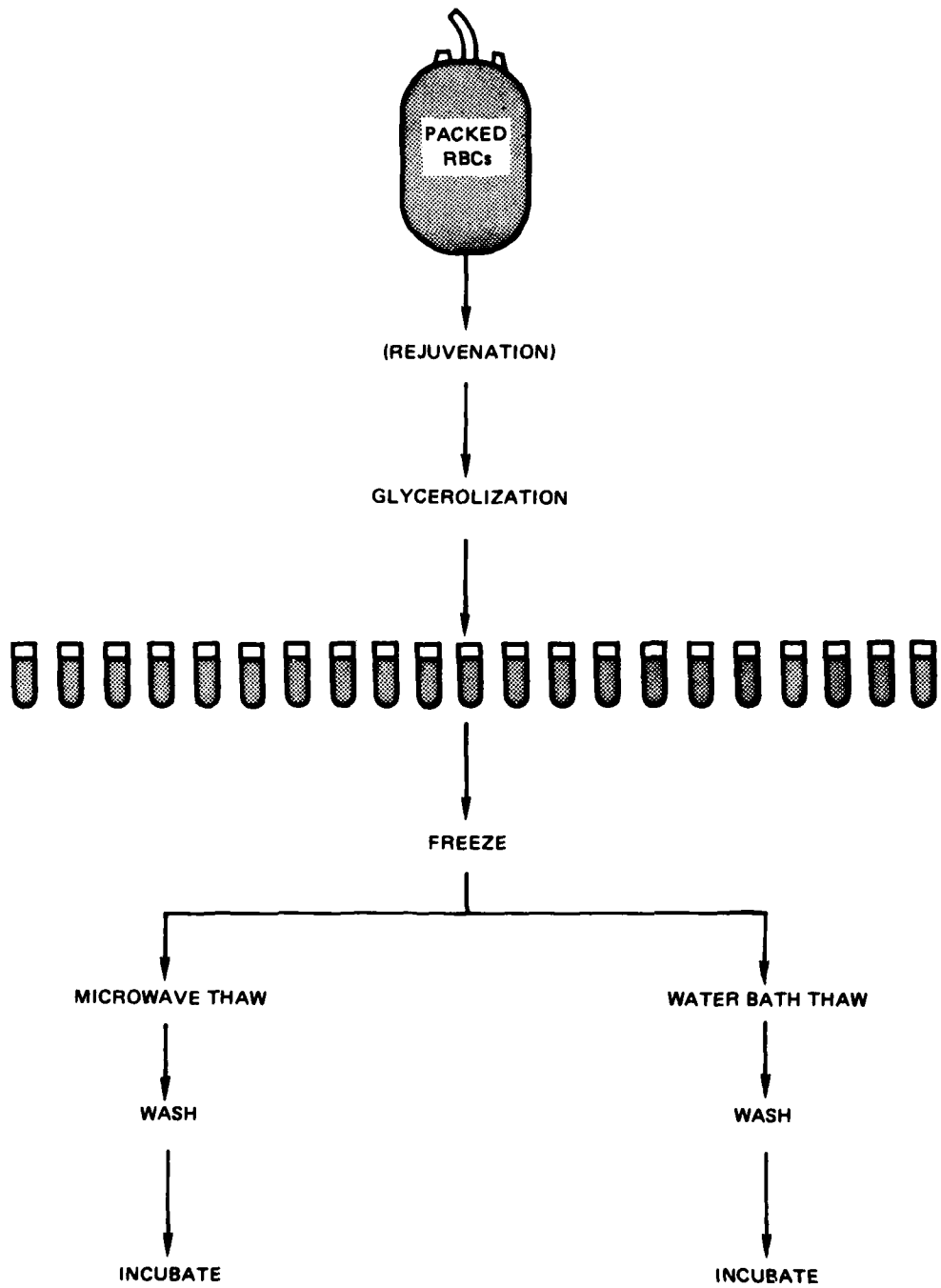


Figure 1. Flow diagram of pRBC treatment sequence.

Units greater than 6 days old show decreased oxygen carrying ability due to low DPG levels. Before these units can be frozen, they should be rejuvenated to improve their oxygen carrying function. Outdated pRBC units were rejuvenated, and all units were glycerolized by procedures developed by Captain C.R. Valeri, MC, USNR (Valeri et al., 1979). These procedures are expected to replace the procedures currently used by Naval Blood Banks. The rejuvenation solution used was PIPA (Cytosol Laboratories, Boston, MA) and contained 100 mM/L pyruvate, 100 mM/L inosine, 100 mM/L disodium phosphate, and 5 mM/L adenine. This solution had an osmolality of 500 mOsm/Kg water and a pH of 7.2. Fifty ml of this rejuvenation solution was added to each outdated pRBC unit. The resulting mixture was incubated at 37°C in a water bath for 1 hour. Fresh pRBC units were not treated with PIPA.

Fresh and rejuvenated RBC (rRBC) units were glycerolized with 6.2M glycerol (57% W/V) in three additions (Table 1). An initial addition of glycerol was made to mechanically agitated red cells. A 5-minute equilibration period with no agitation then followed. A second glycerol volume was added with mixing followed by equilibration for 2 minutes. The third glycerol volume was added with continuous manual agitation. The units were then concentrated by centrifugation at 1250 X g for 7 minutes. The supernatant glycerol was removed, yielding a unit of concentrated glycerolized red cells (gRBCs). The contents of each unit were then aliquoted to sterile, polycarbonate freezer tubes, with approximately 3.5 ml of gRBCs per tube. These tubes were then covered with sterile aluminum foil, labelled, and placed in a -75°C freezer. The polycarbonate tubes were sterilized with the aluminum foil lid in place by placing them in a 200°F oven for 2 hours.

Table 1. Glycerol volumes added to prepare RBCs for freezing (Valeri et al., 1979).

Net Weight of pRBC (grams)	Glycerol First Addition (ml)	Glycerol Second Addition (ml)	Glycerol Final Addition (ml)
90 - 150	25	25	150
151 - 230	50	50	200
231 - 310	50	50	300

THAWING

Microwave thawing and water bath thawing of equal numbers of samples, in sufficient quantity to allow post-wash testing, were performed simultaneously at the beginning of each day of the experiment. When thawing was complete, the WT-RBC samples were combined and the MT-RBC samples were combined. Each was then tested for post-thaw recovery by measuring Hb_t%, Hb_s% and Hct.

Ideally, testing of each individual aliquot should have been performed. However, the capacity of the MT waveguide allowed thawing of only 3.5 ml at a time — an insufficent volume to conduct all desired post-thaw and post-wash tests. Therefore, each day four to five aliquots were thawed by each thawing process, and then combined to supply enough sample for testing.

Water Bath Thaw

Tubes containing frozen gRBCs were removed from the freezer and placed in a 37°C water bath. Care was taken to ensure that the aluminum foil lid did not contact the water of the bath, and that the entire gRBC portion of the tube was submerged. Preliminary testing showed that 5 minutes was the optimal time for water bath thawing of the small samples (i.e., resulted in the highest percent recovery). After 5 minutes, the tubes were removed from the bath, the contents combined in a preweighed sterile 50 ml capacity centrifuge tube, and Hb_t%, Hb_s%, Hct, and specific gravity values were determined.

Microwave Thaw

A Variable Power Source (Gerling-Moore Model 4003), continuously variable from 0 to 2400 watts, was used at 2450 MHz to dry thaw half of the frozen samples. This microwave power source was controlled using a Variable Power Source Controller (Gerling-Moore Model 4006) and a Servo-Control (Gerling-Moore Model 4012). A travelling waveguide with a test tube coupler was used as the applicator to couple the microwave power from the power source into the sample. The top, empty portion of the polycarbonate tube, along with the aluminum foil lid, protruded from the opening into the waveguide. Figure 2 is a block diagram of the microwave thawing system.

The 2450 MHz wave, generated in the power source magnetron, was directed to the sample by the waveguide. The dummy load at the end of the waveguide absorbed most of the energy not absorbed by the sample. A small portion of energy was reflected back toward the magnetron by both the sample and the dummy load. The tuner situated between the sample and the power source allowed adjustment of this reflected power to zero by compensating for any impedance mismatch in the load system. To further protect the magnetron from reflected power, a 3-port circulator (Gerling-Moore Model 4065) was placed before the power source to divert any reflected power to the third port where the energy was absorbed by a second dummy load. Meters were inserted between the 3-port circulator and the tuner to allow visual monitoring of forward and reflected power. A cooling system pumped water through the dummy loads and the power source when the microwave heating unit was in operation.

Preliminary thawing studies indicated that the microwave thawing inside the waveguide was not uniform (i.e., the sample thawed on one side first). Since nonuniform microwave heating has a high potential for thermal runaway and therefore cell damage (see Potential Advantages and Disadvantages of Microwave Thawing), this problem had to be corrected. Causes of nonuniform microwave heating are nonuniformities in the sample composition, the sample shape, and/or the microwave field. Nonuniformities in sample composition may be due to settling of the cells during the time between being placed in the -75°C freezer and being frozen. This settling is minimal and difficult to eliminate; therefore, no attempt was made to correct for nonuniformities in sample composition. The round-bottom tube used in the waveguide was a source of shape nonuniformity, but since the portion of the sample in the bottom of the tube was not the portion of blood thawing first, the tube was not replaced.

The presence of the sample in the microwave field causes reflections of the normally uniform field in the waveguide due to electrical discontinuities. These reflections result in a nonuniform field at the sample. To minimize

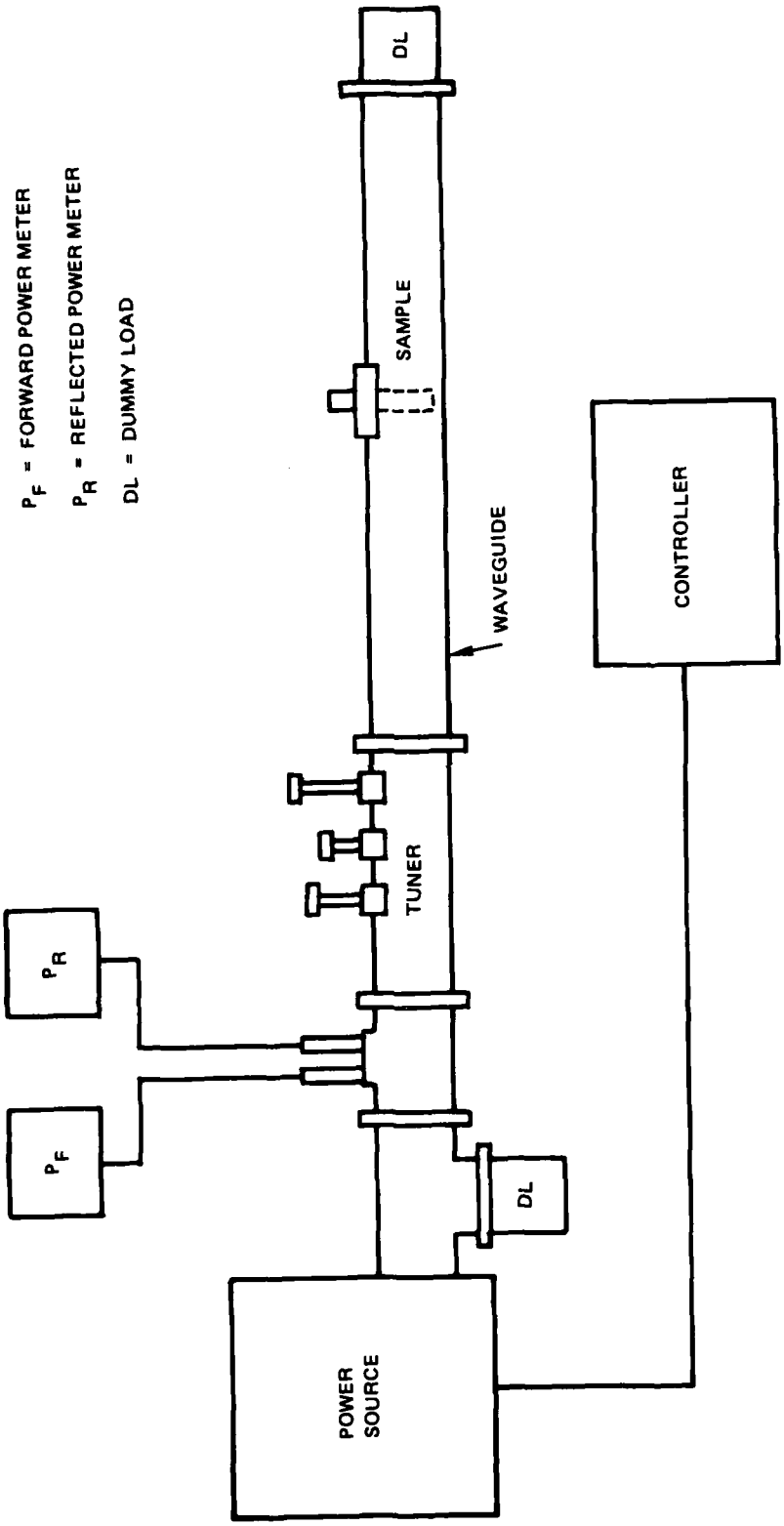


Figure 2. Block diagram of the microwave thawing system.

reflections near the sample, the discontinuity was eliminated by packing SiO_2, a material having electrical properties similar to frozen gRBCs, into the waveguide on either side of the sample. Using this modification, the thawing of samples was better, but still not completely uniform. Finally, a constant manual rotation (approximately 30 revolutions per minute) of the sample during microwave heating was attempted, and produced acceptable thawed samples.

After these modifications were made, the following protocol was used to thaw all MT samples. A 3.5 ml frozen sample was removed from the freezer and inserted into the coupler. The microwave was turned on (preset to 300 watts) and manual rotation was started when the tube had warmed sufficiently to rotate easily (5-10 seconds). Approximately 45 seconds later, the reflected power began to rise. This change in reflected power signalled a change in the blood sample from a frozen to a liquid state. To eliminate any localized overheating, the microwave was turned off when the reflected power reached 20 watts. If examination showed that parts of the sample were still frozen, the tube was reinserted in the coupler and a pulsing technique was used to completely thaw the sample without overheating the already thawed portions. For pulsing, the microwave was turned on for 2 seconds (with rotation of the tube) then off for 10 seconds to allow the heat to equilibrate throughout the sample. This was continued until the sample flowed freely when the tube was tilted to one side. During the 10-second off period, the sample was examined to determine if thawing was complete. Thawing usually required three pulse periods. Approximately 90 seconds were required overall to thaw one tube of frozen RBCs in the waveguide.

For each experiment, four to five tubes of frozen RBCs were thawed, one at a time, using this method. Their contents were combined in a preweighed, sterile, 50 ml-capacity centrifuge tube. Hb_t%, Hb_s%, Hct, and specific gravity were measured.

WASHING

The washing procedure used was a modification of one developed by C.R. Valeri et al. (1979) which varies only slightly from the procedures currently used in blood banks. Washing required two different solutions. Solution A, used in the first stage of washing, was 12% NaCl, manufactured by Fenwal Laboratories.

Solution B, used in the final stages of washing, was a NaCl, dextrose, phosphate buffer solution. Dr. Valeri's Solution B consisted of 0.9% NaCl, 0.2% dextrose, 25 meg/L disodium phosphate, and had an osmolality of about 340 mOsm/Kg water with a pH of 6.8 (Valeri et al., 1979). The wash solutions available on the market are also either buffered with a pH of approximately 6.8 or not buffered at all (pH 6.0 - 6.5). These wash solutions are not suitable for metabolism studies because of their low pH. In vivo, the pH of blood is approximately 7.4 (Darrow, 1964), which is the working pH desired for metabolism studies. Any pH above or below 7.4 may inhibit RBC metabolism. Therefore, an isotonic buffered NaCl-dextrose solution, able to hold a pH as close to 7.4 as possible through 6 hours of RBC metabolism, was developed and used as a wash solution.

Solution B in this study was composed of 0.81% NaCl, 0.18% dextrose, 0.08% sodium phosphate monobasic, and 0.40% sodium phosphate dibasic. the pH

of the heat-sterilized solution was adjusted to 8.4 by addition of a predetermined amount of concentrated, sterile NaOH. This high wash solution pH was required to establish an initial pH of 7.6 in the washed RBC sample. The hematocrit of the washed sample was adjusted low, between 20 and 30%, to limit the production of lactate which would lower the pH too quickly during the 6 hour post-wash incubation.

Solution B was established after several months of experimentation with different combinations of NaCl, dextrose, sodium phosphate monobasic and sodium phosphate dibasic. Most of the alterations involved increasing the buffering capability while maintaining an osmolality around 340 mOsm/Kg water. Preliminary experiments with washed RBCs employed nonsterile wash solutions. These RBC samples showed obvious contamination after 6 hours of study, and their data are not included in this report.

The actual washing procedure is outlined in Table 2 and discussed here. The volumes listed below are in ml per gram thawed RBCs (thRBCs). Washing began by aseptically adding 0.15 ml/gm of solution A (12% NaCl) while swirling the cells. After a 2-minute equilibration period, 0.31 ml/gm of solution B was added with swirling. Two minutes later, 0.46 ml/gm of the same solution was added. The cells were allowed to equilibrate 2 minutes and were then centrifuged 5 minutes at 2500 rpm using an IEC Model 428 centrifuge and Model 215 rotor. The supernatant was carefully removed by sterile pipet to a preweighed beaker. The cells were gently resuspended by swirling, and 1.2 ml/gm of the same solution B was added while agitating the cells. The blood was centrifuged again at 2500 rpm for 5 minutes, and the supernatant carefully removed by pipet to the beaker. This process was repeated two more times. After the removal of the last supernatant, the Hct of the cells was adjusted to approximately 25% by addition of 0.5 ml/gm of solution B. The samples were weighed and approximately 5 ml from both MT- and WT-washed RBCs (wRBCs) were set aside for zero-hour testing which began immediately. The two tubes of the remaining wRBCs (approximately 15 ml each) were capped and placed in a 37°C water bath. The beakers containing the wash waste were reweighed, and specific gravity and Hb_t% measurements were performed.

VIABILITY TESTS

Packed red blood cells are typically transfused to replenish a loss in the oxygen carrying capacity of a patient. The ability of pRBC to do this is reduced when:

1. RBCs are destroyed in vitro during storage and processing;
2. RBCs are destroyed in vivo in the first 24 hours post transfusion; or
3. Viable RBCs have a decreased oxygen-carrying ability.

Destruction of RBCs in vitro was measured as percent recovery (%R) and percent hemolysis (%H). The potential for in vivo survival was determined in vitro by:

1. Monitoring the rate of RBC anaerobic glycolysis as represented by decreased glucose and increased lactate and pyruvate concentrations in the suspending medium over time,

Table 2. Small sample wash procedure.

Procedure	Solution	ml Solution/gram thRBC
First addition	A	0.15
Two minute equilibration		
Second addition	B	0.31
Two minute equilibration		
Third addition	B	0.46
Two minute equilibration		
Five minute centrifugation Remove supernatant		
Fourth addition	B	1.2
Five minute centrifugation Remove supernatant		
Fifth addition	B	1.2
Five minute centrifugation Remove supernatant		
Sixth addition	B	1.2
Five minute centrifugation Remove supernatant		
Seventh addition	B	0.5

Solution A: 12% NaCl

Solution B: 0.81% Nacl, 0.18% dextrose, 0.08% sodium phosphate monobasic, 0.40% sodium phosphate dibasic

2. Measuring the cellular ATP concentration over time, and

3. Measuring the loss of cells over time, expressed as %H.

The ability of the intact RBCs to carry and release oxygen was determined by three factors:

1. The amount of hemoglobin present, expressed in terms of mean cellular hemoglobin (MCH) or mean cellular hemoglobin concentration (MCHC),

2. The RBCs capability of releasing oxygen to the tissues, measured by its 2,3-DPG production and

3. The cellular ability to maintain the hemoglobin molecule in the reduced, functional state, measured by the GSH concentration.

Total Hemoglobin Concentration (Hb_t%)

Total Hb concentration was used in the calculations of %H, %R, and cellular Hb concentration (Hb_c%). Total Hb in grams per 100 ml of solution (gm%) was measured using the cyanomethemoglobin technique first proposed by W.C. Stadie in 1920 (Sigma Chemical Company, 1976c) and available in kit form (#525) from Sigma Chemical Company, (St. Louis, MO). In this test, Drabkins reagent, containing potassium cyanide and potassium ferricyanide, oxidizes hemoglobin to methemoglobin which is then converted to cyanomethemoglobin. The concentration of this latter Hb derivative is determined by measuring its absorbance at 540 nm. A calibration curve prepared from Hb standards (Sigma #525-18) was used to interpolate Hb_t% in unknown solutions.

To determine Hb_t% of wRBC or whole blood (whRBC), 0.02 ml of the sample was added to 5 ml of Drabkins reagent. To determine Hb_t% of more concentrated samples (pRBC, gRBC, rRBC or thRBC), 0.01 ml of the sample was added to 5 ml of Drabkins reagent and the Hb_t% read from the calibration curve was doubled.

The Hb_t% measurement for supernatants (sp) from glycerolization and wash procedures was performed using volumes of sp and Drabkins reagent different from those used in preparation of the standard curve. The actual Hb_t% of the sp solution was calculated by multiplying the measured Hb_t% by appropriate correction factors for the unusual dilutions.

$$Hb_t\%_{actual} = \frac{.02}{5.02} \left(Hb_t\%_{measured}\right) \frac{\text{ml sp + ml Drabkins}}{\text{ml sp}} \qquad (1)$$

Hematocrit (Hct)

Hematocrit is the volume percent of a blood solution that is composed of red blood cells. This value was needed to determine Hb_s%, Hb_c%, %H and %R. A small sample of the blood was drawn up into a capillary tube and spun at 11,800 rpm for 5 minutes (whRBC and wRBC) or 7 minutes (pRBC, gRBC, rRBC and thRBC), using a microhematocrit centrifuge (Model 0556, Clay Adams, Parsippany, NJ). (This has recently been changed in our laboratory to 3 minutes and 5 minutes, respectively.)

Supernatant Hemoglobin Concentration (Hb_s%)

Supernatant Hb is the Hb found free in solution unassociated with intact cells. It was used in the calculations of Hb_c%, %H, and %R. A variation of Lewis' (1965) o-tolidine method of measuring Hb_s% in grams per 100 ml of supernatant was adopted, using procedures developed by the Hematology Laboratory, Naval Regional Medical Center, San Diego. In this test, o-tolidine is used as the hydrogen donor in a peroxidase reaction requiring hemoglobin. The absorbance of the primary oxidation pigment of o-tolidine is read at 630 nm on the spectrophotometer exactly 10 minutes after initiation of the reaction. The production of this color is directly related to the Hb concentration present. A standard was run with each experiment because of the high sensitivity of this test to slight variations in procedure or reagents.

Because of high concentrations of Hb in the supernatant of experimental samples (as compared to fresh whole blood, for which the test was devised), initial dilutions (dilution 1) of the samples were made in isotonic solutions. A known volume of blood was added to a known volume of isotonic solution, gently swirled, and centrifuged to remove the RBCs. The supernatant was stored in the refrigerator for later testing. The actual Hb_s% test procedure used was as follows:

A frozen standard hemoglobin solution of approximately 7.5 g% (prepared earlier from lysed RBCs) was thawed, and the Hb_t% was measured by the Drabkins method. A volume of this standard was diluted in each of the isotonic solutions used in the samples to a concentration of approximately 20 mg% (mg per 100 ml solution). The color intensities of the regrigerator-stored sample supernatants were visually compared with those of the diluted standards. Further dilutions (dilution 2) of the samples were performed as necessary to bring their Hb_s% to approximately that of the standard.

Two working solutions were freshly prepared before each test: 10% glacial acetic acid and 1% hydrogen peroxide. The o-tolidine solution was prepared in advance and stored in the refrigerator for no longer than 8 weeks (0.25 grams o-tolidine with 90 ml glacial acetic acid and 10 ml distilled water).

One ml of the o-tolidine solution was added to a 15 ml tube for each standard and sample. One-tenth of a ml (0.05 ml for glycerol based solutions) of the standard or sample was added to this tube and was allowed to equilibrate at least 2 minutes. This test required reading of the solution exactly 10 minutes after the reaction, so initiation of the reaction for each sample or standard was staggered at precisely-timed intervals to allow uninterrupted reading of the end product exactly 10 minutes later.

The reaction was initiated by adding 1 ml of the 1% H_2O_2 solution, followed by gentle swirling. Exactly 10 minutes later, the solution was diluted with 10 ml of the 10% glacial acetic acid solution to allow reading of the absorbance at 630 nm, using water to zero the spectrophotometer.

Calculations were as follows:

1. Concentration of diluted Hb standard

$$\text{dstd g\%} = \frac{\text{Hb}_t\% \times (\text{ml std})}{(\text{ml iso})} \times 100 \qquad (2)$$

2. First dilution of blood sample

$$df_1 = \frac{(\text{ml iso}) + (\text{ml RBC})(1-(\text{Hct} \times 10^{-2}))}{(\text{ml RBC})(1-(\text{Hct} \times 10^{-2}))} \qquad (3)$$

3. Dilution of supernatant of dilution 1

$$df_2 = \frac{(\text{ml sp}_1) + (\text{ml iso})}{(\text{ml sp}_1)} \qquad (4)$$

4. Supernatant Hb of sample

$$\text{Hb}_s\% = \frac{\text{dstd g\%}}{A_{std}} (A_{unk}) \frac{\text{ml std tested}}{\text{ml sp}_2 \text{ tested}} (df_1)(df_2) \qquad (5)$$

where:

A_{std} = absorbancy of standard
A_{unk} = absorbancy of unknown
std = undiluted standard
dstd = diluted standard
iso = isotonic solution
df = dilution factor
RBC = undiluted blood sample
sp_1 = supernatant of 1st dilution
sp_2 = supernatant of 2nd dilution
g% = grams per 100 ml solution
Hct = hematocrit

Cellular Hemoglobin Concentration ($Hb_c\%$)

This value was used in the normalization of the metabolic data, and in calculating MCH and MCHC. Supernatant Hb (Hb_s) per 100 ml of supernatant ($Hb_s\%$) was converted to Hb_s per 100 ml solution by multiplying by the volume of supernatant (sp) in solution (sn).

$$\frac{Hb_s}{100 \text{ ml sn}} = \frac{Hb_s}{100 \text{ ml sp}} \times \frac{(100 - Hct) \text{ ml sp}}{100 \text{ ml sn}} = (Hb_s\%)\ (1 - (Hct \times 10^{-2})) \quad (6)$$

This value was subtracted from the total Hb in 100 ml of solution ($Hb_t\%$) to yield Hb in 100 ml of RBC ($Hb_c\%$).

$$Hb_c\% = Hb_t\% - [Hb_s\%\ (1 - (Hct \times 10^{-2}))] \quad (7)$$

Red Blood Cell Count (RBC#)

The RBC#, given in millions of cells per mm^3 of solution, was used in the determinations of mean cellular volume (MCV) and MCH. Counts were performed using the Clay Adams HA-5 Hematology Analyzer Model 2550. The HA-5 counts cells by detecting small current changes induced by the passage through a small orifice of non-conducting blood cells suspended in a conducting diluent. White as well as red blood cells are counted, but in normal blood samples the white cell count is relatively small and may effectively be ignored. Only a very small sample volume (25 μl) per 6.5 ml of diluent (modified Eagle's solution) is necessary. Counts were performed on pre-glycerolized and post-thaw washed blood samples. Counts could not be performed on glycerolized samples due to conductivity problems encountered with the use of glycerol as a diluent.

Percent Recovery (%R)

The freezing, thawing and washing procedures unavoidably lysed some RBCs. The %R measurement indicates the percentage of cells that survived each of the processes.

In this study, the location of the Hb was used to determine the condition of the cells following a particular procedure. Hemoglobin is either located in the cell or in the supernatant, the latter if a cell has lysed. A comparison of the Hb associated inside the cell (Hb_c) before and after a procedure then indicated the percentage of cells that survived.

Freeze-thaw percent recoveries were calculated from measurements of pre-freeze $Hb_s\%$ and Hct, and post-thaw $Hb_t\%$, $Hb_s\%$ and Hct.

$$Hb_{c\text{-}post}\% = Hb_{t\text{-}post}\% - (1 - (Hct_{post} \times 10^{-2}))(Hb_{s\text{-}post}\%) \quad (8)$$

$$Hb_{c\text{-}pre}\% = Hb_{t\text{-}post}\% - (1 - (Hct_{pre} \times 10^{-2}))(Hb_{s\text{-}pre}\%) \quad (9)$$

$$\%R_{freeze\text{-}thaw} = \frac{Hb_{c\text{-}post}\%}{Hb_{c\text{-}pre}\%} \times 100 \quad (10)$$

Thawed RBCs, for an unknown reason, have a slightly higher $Hb_t\%$ than the pre-freeze values (Travenol Laboratories, Inc., 1973). Therefore, $Hb_{t\text{-}post}\%$ was used for both pre and post values on freeze-thaw recoveries.

In wash percent recovery calculations, "pre" referred to measurements taken pre-wash (equivalent to post-thaw), and "post" referred to measurements taken post-wash. The wash percent recoveries were more difficult to calculate since the volumes and concentrations of the RBC samples were different between pre- and post-wash, and because some of the blood was lost on the sides of glassware during the wash procedure. To accommodate for the different volumes and concentrations, the measured relative values of hemoglobin concentrations ($Hb_t\%$ and $Hb_s\%$) were converted to actual values (Hb_t and Hb_s) by multiplying by sample volume. The blood lost during the procedure was ignored by estimating $Hb_{t\text{-}pre}$ from post values:

$$Hb_{t\text{-}pre} = Hb_{t\text{-}post} + Hb_{t\text{-}waste} \tag{11}$$

where $Hb_{t\text{-}waste}$ was the actual weight of hemoglobin in the wash waste (calculated from $Hb_{t\text{-}waste}\%$ x ml waste). Therefore,

$$Hb_{c\text{-}post} = Hb_{t\text{-}post} - (1 - Hct_{post} \times 10^{-2}))(Hb_{s\text{-}post}) \tag{12}$$

$$Hb_{c\text{-}pre} = Hb_{t\text{-}post} + Hb_{t\text{-}waste} - (1 - Hct_{pre} \times 10^{-2}))(Hb_{s\text{-}pre}) \tag{13}$$

$$\%R_{wash} = \frac{Hb_{c\text{-}post}}{Hb_{c\text{-}pre}} \times 100 \tag{14}$$

Percent Hemolysis (%H)

This value reflects the percentage of cells that are lysed in a particular sample at any one time. It does not compare the pre and post procedural condition of an RBC sample as does %R. The free Hb in the supernatant ($Hb_s\%$) times $(1 - (Hct \times 10^{-2}))$ represented the number of cells that had been lysed. The total Hb ($Hb_t\%$) represented the total number of cells if no cells had been lysed. The %H was the ratio of these two values.

$$\%H = \frac{Hb_s\% \ (1 - (Hct \times 10^{-2}))}{Hb_t\%} \times 100 \tag{15}$$

Red Blood Cell Indices

Mean cellular volume (MCV), mean cellular Hb (MCH), and mean cellular Hb concentration (MCHC) denote average morphological characteristics of the RBCs.

$$MCV = \frac{Hct \times 10}{RBC\#} \qquad \text{Average values: } 80 - 92\mu^3 \tag{16}$$

$$MCH = \frac{Hb_t\% \times 10}{RBC\#} \qquad \text{Average values: } 27 - 31 \mu\mu g \qquad (17)$$

$$MCHC = \frac{Hb_t\% \times 100}{Hct} \qquad \text{Average values: } 32 - 36\% \qquad (18)$$

(All average values obtained from Brown, 1976.)

The MCH and MCHC values reflected any potential loss of cellular Hb. The MCV value was used to observe the effect of a treatment on cell size.

Metabolism

Red blood cells undergo anaerobic metabolism to break glucose down to lactate via the Embden-Meyerhoff pathway shown in Figure 3. This multi-stepped process produces four important products:

1. Adenosine-5'-triphosphate (ATP), powering the sodium-potassium pump and maintaining the biconcave shape of the cell,

2. Reduced nicotinamide adenine dinucleotide (NADH), reducing oxidized Hb back into its functional state,

3. Reduced glutathione (GSH), also maintaining the Hb in its reduced, functional state, and

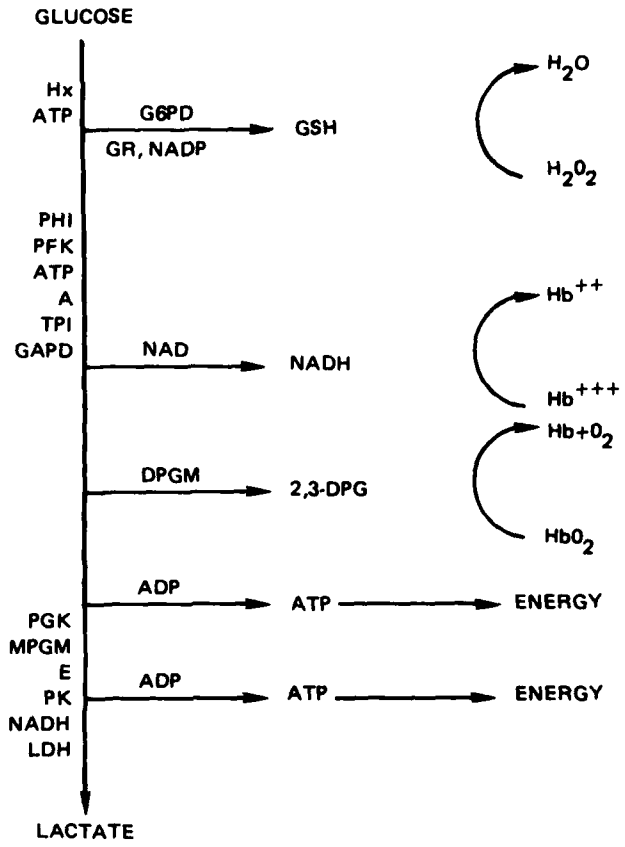
4. 2,3-diphosphoglycerate (2,3-DPG), altering the oxygen affinity for Hb to accommodate tissue demand.

Adenosine triphosphate, DPG, and GSH were directly assayed in each sample. Glucose and lactate were assayed to approximate the rate of glycolysis.

The concentration of methemoglobin (metHb), which is the oxidized, non-functional form of Hb, was to be measured to monitor the effectiveness of GSH and NADH. However, preliminary attempts to assay metHb in blood samples (using methods described by Brown, 1976) showed a strong dependence of the measured metHb value on the time allowed for reaction. Therefore, metHb measurements were eliminated from the final analysis. Future studies may employ this measurement, but should do so only under strict timing practices.

Adenosine triphosphate was assayed using T. Bucher's phosphorylation reaction coupled with a dephosphorylation/oxidation reaction, as modified by H. Adams in 1963 (Sigma Chemical Company, 1974b). In this assay procedure, ATP acts as a phosphate donor in a two step reaction converting NADH to NAD. The decrease in absorbance of NADH (measured at 340 nm) reflects the original ATP concentration. A standard curve was prepared using various dilutions of ATP. Unknown ATP concentrations were read from this curve using the absorbancy changes produced by the experimental samples. Average ATP values in fresh whole blood are 3.65 - 4.45 μmol/gHb (Sigma, 1974b).

The concentration of 2,3-DPG was measured using the same dephosphorylation/oxidation reaction as the ATP assay, but using 2-phosphoglycolic acid as a stimulator, as described by Lowry in 1964 (Sigma Chemical Company,



ABBREVIATIONS

A	= aldolase
ADP	= adenine diphosphate
ATP	= adenine triphosphate
2,3-DPG	= 2,3-diphosphoglycerate
DPGM	= diphosphoglyceromutase
E	= enolase
GAPD	= glyceraldehyde phosphate dehydrogenase
G6PD	= glucose-6-phosphate dehydrogenase
GR	= glutathione reductase
GSH	= reduced glutathione
Hb^{++}	= hemoglobin reduced
Hb^{+++}	= hemoglobin oxidized
Hx	= hexokinase
LDH	= lactic dehydrogenase
MPGM	= monophosphoglyceromutase
NAD	= nicotinamide adenine dinucleotide
NADH	= nicotinamide adenine dinucleotide, reduced
NADP	= nicotinamide adenine dinucleotide phosphate
PFK	= phosphofructokinase
PGK	= phosphoglycerate kinase
PHI	= phosphohexose isomerase
PK	= pyruvate kinase
TPI	= triose phosphate isomerase

Figure 3. Glycolytic pathway of the red blood cell. Shown in the center are important by-products, and on the left, the necessary enzymes.

1974a). In this three step procedure 2,3-DPG is hydrolyzed to 3-phosphoglycerate (3-PGA). This latter compound is phosphorylated by ATP to produce 1,3-diphosphoglycerate (1,3-DPG) which oxidizes NADH. The decrease in NADH absorbance at 340 nm reflects the concentration of 2,3-DPG originally present. Unknown 2,3-DPG concentrations were read from a standard curve which was prepared using known 2,3-DPG concentrations. Expected concentrations of 2,3-DPG in fresh whole blood range from 10.5 to 16.2 μmol/gHb (Sigma, 1974a).

Reduced glutathione reacts with 5,5'- Dithiobis-(2-nitrobenzoic acid) (DTNB) to form oxidized glutathione. The intensity of the yellow color of the oxidized glutathione, measured at 412 nm, indicates the concentration of GSH originally present. Blood samples from units 15-19 were tested using reagents in kit form from Princeton Biomedix Inc., while samples from units 20-23 were tested using fresh reagents as described in Beutler et al., 1963. Both techniques are essentially the same, using the above reaction to produce oxidized glutathione. Unknown GSH concentrations were read from a standard curve prepared using three dilutions of GSH (Biomedix #500420). Normal values of GSH in fresh blood range from 78 to 116 mg/100 ml RBC (Princeton Biomedix, Inc, 1976).

Glucose was measured by Raabo and Terkildsen's two step oxidation reaction initiated by glucose and forming a final product absorbing at 450 nm (Sigma, 1976a). Glucose oxidase converts glucose to gluconic acid and hydrogen peroxide by the addition of oxygen and water. The hydrogen peroxide oxidizes o-dianisidine to a product absorbing at 450 nm. The absorbancy at this wavelength determines the initial glucose concentration. A standard of 1 mg glucose/ml was run with each sample. The glucose concentration of the sample was calculated by:

$$\text{Glucose (mg/100 ml)} = \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{standard}}} \times 100 \qquad (19)$$

A calibration curve was prepared using standard glucose solutions ranging from 50 to 300 mg% to assure linearity of the reaction at high glucose concentrations. The normal range of glucose concentration in whole blood of a fasting adult is 50 to 90 mg% (Sigma, 1976a).

Lactate was assayed using a procedure developed in 1968 by R.J. Henry which observes the conversion of lactate to pyruvate with subsequent reduction of nicotinamide adeninine dinucleotide (NAD). This reaction is catalized by lactate dehydrogenase (Sigma Chemical Company, 1976b). The increase in absorbance at 340 nm, indicating the reduction of NAD to NADH, is proportional to the lactate concentration. The actual concentration was read from a standard curve prepared from five dilutions of a lactic acid standard. The normal range for lactate in fasting venous blood is 0.3 to 1.3 mmol/liter (Sigma, 1976b).

Pyruvate concentrations were determined in a few samples. Pyruvate was measured using the reverse of the above reaction, producing lactate and NAD. The decrease in absorbance at 340 nm, reflecting the oxidation of NADH to NAD, represents the initial pyruvate concentration. A standard curve was prepared

from solutions containing known pyruvate concentrations. Normal values of pyruvate in fasting venous blood are 0.03-0.08 mmol/liter (Sigma, 1976b).

Each of these metabolic indicators was assayed and the pH of the sample read immediately after washing the cells and then every 2 hours up to 6 hours post-wash. The values for glucose, lactate, pyruvate, ATP, 2,3-DPG, and GSH were recorded per gram of Hb_c to normalize the data for different cell concentrations in the samples.

The hemoglobin-oxygen dissociation curve was measured on washed samples from units 22 and 23 to determine proper oxygen uptake and release from hemoglobin. The curves were generated by a HEMO-O-SCAN Oxygen Dissociation Analyzer. This system uses a dual-wavelength spectrophotometer to monitor oxyhemoglobin (oxyHb) concentration, and a Clark oxygen electrode to measure partial pressure of oxygen (pO_2). The resulting dissociation curve is recorded on an X-Y recorder.

For the actual procedure, a 2 μl sample of blood was placed on a cover glass and covered with a gas permeable membrane. The resulting thin film of blood minimized the time required for thorough oxygenation. The sample was placed in a compartment inside the HEMO-O-SCAN analyzer and was allowed to equilibrate to a high humidity at 37°C, as provided by the flow of warmed water around the compartment. Equilibration was made to 100% oxyHb with a 25% O_2, N_2 balance gas mixture. The X-Y recorder was adjusted to mark 100% oxyHb. This equilibration period was usually used to bring bicarbonate buffered samples to a partial pressure of CO_2 at 40 mm Hg. Since the samples in these experiments were buffered with phosphate buffers, CO_2 gas mixtures were not used. After equilibration, the sample was deoxygenated by purging the compartment with 100% N_2 for 5 minutes. These 0% oxyHb and zero pO_2 points were marked on the X-Y recorder. The surrounding pO_2 was then gradually increased by addition of 25% O_2, N_2 balance gas, and the corresponding %oxyHb recorded until 100% oxyHb was achieved, in about 15 minutes. P50 refers to the pO_2 required to produce a 50% oxyHb fraction. These p50 values were measured from the curves and recorded.

Contamination studies were performed on 0 and 6 hour samples from units 19 to 23. A 0.5 ml volume of blood was placed in a sterile 10 cm diameter petri dish by sterile pipette. Tryptic Soy Agar (Difco Laboratories), melted and cooled to just above solidification (30°C), was added to the dish to leave about a 1 cm layer of agar. The blood was mixed through the agar by swirling. This mixture was then incubated at 37°C for 2 days and examined for bacterial colonies.

STATISTICAL TEST

The null hypothesis (H_o) addressed in this study assumes no difference in morphologic and metabolic parameters measured between microwave and water bath thawed blood samples. The null hypothesis will be rejected if F ratio statistics exceed critical values at the $P<0.05$ significance level. In cases where H_o is rejected, the alternate hypothesis (that a significant difference in morphologic and metabolic blood parameters did exist) will be adopted.

A two-way analysis of variance (ANOVA) was performed on a Univac 1100 computer using the Statistical Package for the Social Sciences (Nie, N.H., et

al., 1975). The SPSS statistical treatment assumed a fixed-effect model (the main effects tested were thawing treatment and blood units) and was capable of testing experimental models with unequal cell frequencies. Three ANOVA testing approaches were available in the SPSS software package where data with unequal cell frequencies could be treated. In this study, the classical approach was used. This approach assumes that factors do not have a known causal order. Interaction effects were tested first and if these were not significant, main effects were subsequently tested.

RESULTS

In this section, data are shown for blood samples at different stages in the freeze-thaw processing: whRBC, pRBC, rRBC, gRBC, thRBC, and wRBC. Samples from eight units of blood, labelled 15, 17, 18, 19, 20, 21, 22, and 23, were analyzed for differences in microwave versus water bath thawing in this study. Results for thRBCs and wRBCs are presented showing the values from each unit individually, and the overall average value from all eight units to determine differences in the thawing method.

FRESH WHOLE BLOOD

Fresh whole blood was periodically collected (using heparin as the anticoagulant) from volunteer donors to determine normal ranges in our laboratory for each of the testing methods. The results are shown in Table 3. The data collected from these tests indicate acceptable testing procedures (i.e., the findings fell within the expected normal range).

Note that the units used in Table 3 to represent fresh whole blood values for glucose, lactate, pyruvate, ATP, DPG, and GSH were not used in further tables. Because of the varying cell concentrations encountered through the freeze-thaw processing, the units for the metabolic parameters in other than fresh whole blood were standardized for the amount of Hb_c in the sample.

PACKED RED BLOOD CELLS

Packed red blood cells were tested for morphologic and metabolic characteristics. These values are shown in Table 4. All units were collected in CPD. As mentioned earlier, two units (15 and 19) were obtained fresh (less than 6 days old) while the other units were obtained outdated (greater than 21 days old). No pRBC data were collected on Unit 15. Average values listed were calculated from all data collected, except on parameters glucose, lactate, and DPG. Since the concentration of these metabolites is highly dependent on the age of the blood, averages are expressed for outdated units only.

As expected, a difference is observed between the fresh and outdated units in glucose and lactate concentrations. The low glucose and high lactate in the outdated units reflect the occurrence of anaerobic metabolism during storage. The extremely low DPG levels in outdated units indicate the effect of low pH on DPG production. Levels of DPG have been previously observed to decrease as a function of lowering pH (Wintch and James, 1977; Valeri, 1975). These near-zero values, combined with the low DPG concentration in the 4 day old unit, exemplify the need to freeze RBC units before they are 6 days old.

Both ATP and GSH concentrations are also low compared to normal levels (3.65 to 4.45 μmol/gmHb and 5.6 to 7.6 μmol/gmHb) respectively, Beutler et al., 1963). These low values are probably due to a low pH with inhibited metabolism.

Table 3. Fresh whole blood, morphologic and metabolic characteristics.

		Hb_t% (gm%)	Hct (%)	RBC# ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)	MCV (μ^3)	MCHC (%)	MCH ($\mu\mu g$)	Glucose (mg%)	Lactate (mg%)	Pyruvate (mg%)	ATP (μmol/100ml)	GSH (mg% RBC)	DPG (μmol/ml)	P_{50} (mm Hg)
NORMAL RANGE	Male	14-17	42-52	4.2-5 4											
	Female	12-15	36-46	3.6-5.0											
	Male & Female				5-10	80-97	32-36	27-31	50-90	3-12	0.3-0.7	38-62	78-116	1.6-2.6	26.0
SOURCE*		1	1	1	1	1	1	1	3	3	3	2	2	2	2
DONOR	CA	14.4	41.5	4.8	8.1	86.5	34.7	30.0	108.7	11.9	0.5	69.4	84.7	2.4	26.0
	MP (Female)	14.1	40.3	4.7	7.4	85.7	35.0	30.0	93.6	7.8	0.7	61.8	81.1	2.0	30.0
	PH	17.2	45.5	5.8	8.1	78.4	37.8	29.7	94.4	3.8	0.8	64.7	83.3	1.9	28.5
	SL	15.2	43.0	5.2	6.6	82.7	35.3	29.2	94.0	2.2	0.8	63.3	70.1	2.2	26.3
	IS	15.2	44.3	5.7	6.4	77.7	34.3	26.7	103.6	11.3	0.5	66.7	61.7	1.9	26.0
	WL	14.8	42.5	4.8	4.4	88.5	34.8	30.8	94.7	10.2	0.5	73.5	98.0	2.2	26.8
	TL	17.2	47.3	5.6	8.4	84.5	36.4	30.7	111.5	6.3	1.0	73.1	86.5	2.1	27.0
	KS	15.7	44.8	5.6	6.7	80.0	35.0	28.0	87.2	3.9	1.0	69.8	80.1	2.0	27.5
	JD	14.7	42.5	4.9	4.8	86.7	34.6	30.0	89.6	5.8	0.4	67.7	89.1	2.2	26.0
	FB	14.9	41.5	4.9	4.8	84.7	35.9	30.4	82.8	8.7	0.5	58.9	83.2	2.0	27.8
	RK	15.9	44.8	5.1	4.8	87.8	35.5	31.2	85.4	7.2	1.0	67.5	71.7	2.0	25.0
	BD	16.0	45.3	5.1	4.2	88.8	35.3	31.4	81.3	11.1	0.7	66.5	75.7	2.1	26.0
	MH (Female)	15.4	44.8	4.8	6.4	93.3	34.4	32.1	112.9	11.3	1.2	72.5	118.5	2.1	26.0
	NC (Female)	13.4	38.5	4.6	4.2	83.7	34.8	29.1	100.5	6.8	1.1	59.9	80.6	2.2	25.8
	BK	15.5	44.3	5.4	5.6	82.0	35.0	28.7	83.7	5.4	0.8	59.0	98.2	2.2	27.0
AVERAGES	Male n	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Male $\bar{x}$	15.6	43.9	5.2	6.1	84.0	35.4	29.7	93.1	7.3	0.7	66.7	81.9	2.1	26.7
	Female n	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Female $\bar{x}$	14.3	41.2	4.7	6.0	87.6	34.7	30.4	102.3	8.6	1.0	64.7	93.4	2.1	27.3
	Male & Female n	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Male & Female $\bar{x}$	15.3	43.4	5.1	6.1	84.7	35.3	29.9	94.9	7.6	0.8	66.3	84.2	2.1	26.8

*SOURCES

1 Normal ranges were obtained from Brown, 1976.
2 Normal ranges were obtained from the assay kit manufacturer's studies (see text for manufacturer).
3 Normal ranges listed are for fasting individuals and were obtained from the assay kit manufacturer's studies.

Table 4. Packed red blood cells, morphologic and metabolic characteristics.

	Age (days)	%H (%)	Hb_t% (gm%)	Hb_s% (gm%)	Hct (%)	Hb_c% (gm%)	RBC# ($x10^6/mm^3$)	MCV (μ^3)	MCHC (%)	MCH ($\mu\mu g$)	Glucose (μmol/gHb)	Lactate (μmol/gHb)	Pyruvate (μmol/gHb)	ATP (μmol/gHb)	GSH (μmol/gHb)	DPG (μmol/gHb)	pH
Unit																	
17	24		23.4		71.5				32.7								
18	22		28.9		85.0				35.2								
19 (Fresh)	4	0.1	22.4	0.1	71.0	22.4	7.4	96.3	31.5	30.4	59.1	16.7		2.2		6.0	
20	26	0.2	22.9	0.1	70.5	22.9	7.5	94.0	32.4	30.5	2.2	30.7	0.9	2.2		0.0	6.87
21	26	0.5	26.9	0.5	75.5	26.8	7.9	95.2	35.5	33.8	1.9	114.6	1.5	1.8		0.0	6.82
22	23	0.2	26.4	0.2	77.0	26.3			34.2		3.1	130.6	1.9	2.1	0.6	0.7	
23	21	0.2	27.6	0.3	82.0	27.6			33.7		9.2	112.7	0.8	2.3	0.7	0.5	
$\bar{X}$		0.2	25.5	0.2	76.1	25.2	7.6	95.2	33.6	31.6	4.1*	97.2*	1.3	2.1	0.7	0.3*	6.85

*These averages are for the outdated units only.

Table 5. Rejuvenated red blood cells, morphologic and metabolic characteristics.

	%H (%)	Hb_t% (gm%)	Hb_s% (gm%)	Hct (%)	Hb_c% (gm%)	RBC# ($x10^6/mm^3$)	MCV (μ^3)	MCHC (%)	MCH ($\mu\mu g$)	Glucose (μmol/gHb)	Lactate (μmol/gHb)	Pyruvate (μmol/gHb)	ATP (μmol/gHb)	GSH (μmol/gHb)	DPG (μmol/gHb)	pH
UNIT																
17	0.2	19.6	0.1	54.3	19.6			36.1								
18	0.4	24.4	0.3	66.0	24.3			36.7								
20	0.2	21.2	0.1	60.0	21.1	7.1	85.3	35.2	29.6	2.0	140.0	8.3	3.7		6.0	6.80
21	0.7	22.0	0.4	59.0	21.8	6.7	88.3	36.9	32.6	1.4	159.2	7.4	3.7		17.0	6.78
22	0.2	25.3	0.1	67.0	25.3			37.7		3.1	146.6	11.1	3.8	5.9	13.3	
23	0.2	26.7	0.1	72.0	26.7			37.0		6.7	121.9	10.7	3.6	6.5	9.9	
$\bar{X}$	0.3	23.2	0.2	63.1	23.1	6.9	86.8	36.6	31.1	3.3	141.9	9.4	3.7	6.2	11.6	6.79

REJUVENATED RED BLOOD CELLS

Morphologic and metabolic parameters were measured on outdated, rejuvenated RBCs and are presented in Table 5. Metabolic parameters were not measured on rejuvenated units 17 and 18. The purpose of rejuvenation was to increase the DPG levels in outdated RBCs from almost zero to at least 100% of normal. This increase was accomplished in the outdated units studied, as indicated in Table 5. In addition to stimulating DPG production, the rejuvenation process also stimulated ATP and GSH production, again to at least 100% of normal levels. Glucose values were even lower, and lactate values even higher than pRBC values, indicating metabolic activity during the 1 hour of rejuvenation. The cells appear to have decreased slightly in size during rejuvenation as shown from the lower MCV values and higher MCHC values.

GLYCEROLIZED RED BLOOD CELLS

Morphologic measurements were made on freshly glycerolized red blood cells to establish pre-freeze values which were later compared to post-thaw values. The gRBC data for all units studied are shown in Table 6. Many of the Hb_s% values were greater than 0.4 gm%, which is higher than expected. These high values are due to initially high Hb_s percentages in the pRBC units, and to inadequate mixing of the blood during glycerolization. (This problem has since been corrected.) The MCHC values are within normal ranges, indicating no change in cell volume due to the exchange of water for glycerol inside the cell.

Table 6. Glyceralized red blood cells, morphologic characteristics.

	%H (%)	Hb_t% (gm%)	Hb_s% (gm%)	Hct (%)	Hb_c% (gm%)	MCHC (%)
Unit						
15	0.1	21.8	0.8	69.5	21.6	31.0
17	0.3	16.0	0.1	47.5	16.0	33.6
18	0.9	15.9	0.3	46.0	15.8	34.3
19	1.0	16.6	0.5	66.0	16.4	24.9
20	0.5	19.4	0.3	67.5	19.3	28.5
21	0.4	18.7	0.2	66.0	19.6	29.7
22	2.5	23.9	1.8	66.3	23.3	35.2
23	1.7	21.9	1.0	63.0	21.5	34.1
$\bar{X}$	0.9	19.3	0.6	61.5	19.2	31.4

THAWED AND WASHED RED BLOOD CELLS

Data for thawed and washed RBCs were collected from several samples of each of the eight units of blood. After glycerolization, each unit was divided into 3.5 ml samples and frozen. All samples tested from a specific unit

had the same pre-freeze values and should have the same post-thaw values except for variations due to the thaw procedure. Half of the samples from each unit were thawed in a warm water bath, while half were thawed with microwave energy. Groups of samples were then manually washed and incubated for 6 hours to test for metabolic integrity. Appendices A and B list the individual unit averages (data of all samples from each unit combined) for all parameters measured immediately post-thaw, then at 0, 2, 4, and 6 hours post-wash. Averages are first listed for the two thawing procedures (WT and MT), then for the two procedures combined. Appendices C and D show the averages from the combined data of all eight units.

Thawed Red Bood Cells

Currently, the acceptable freeze-thaw percent recovery in blood banks is 97.0% or greater. As shown in Appendices A and B, four of the units in these experiments had unacceptable average percent recoveries (less than 97%) by clinical standards. These low recoveries were a result of both the difference in the test tube sample size compared to the full size units used clinically, and the glycerolization problem discussed in the section, "Glycerolized Red Blood Cells." As expected, Hb_s% and %H values increased in the thawed samples compared to the pre-freeze values (Table 6). Also some of the Hb_t% values increased, for reasons that are unclear at this time (see section on "Percent Recovery (%R)."

Washed Red Blood Cells

The zero hour %R values (Appendices) were low due to the manual washing techniques used in these experiments. Manual washing of small samples caused more RBC manipulation, longer washing times, greater variability in added volumes of wash solution, and greater variability in mixing rates, each potentially causing lower %R values. The low Hct and Hb_t% values at zero hour reflect the attempt to slow down metabolic waste product build-up (see section on "WASHING"). Due to the high osmolality of the wash solution, the cells were smaller than average in size at zero hour, as indicated by the relatively high MCHC and low MCV values. The MCH values were normal.

The dc/dt parameters given in the Appendices refer to rates calculated to demonstrate the decrease in glucose and the increase in lactate over time. The data for zero hour is the change in concentration of either glucose or lactate from 0 to 2 hours post-wash, divided by 120 minutes. The data for 2 and 4 hours are for the rate of concentration change between 2 and 4 hours and between 4 and 6 hours, respectively. And the data for 6 hours is the overall rate of change between 0 and 6 hours post-wash.

Four pairs (WT and MT) of thawed and washed samples (two each from Units 22 and 23) were tested for proper hemoglobin function at 0 hour and again at 4 hours post-wash. This function was measured by producing oxygen-dissociation curves for each sample. The curves for a 4 hour, WT and MT sample pair are shown in Figure 4. Table 7 lists the p50 values from each curve. This value is the partial pressure of oxygen necessary for 50% of the Hb to be oxygen saturated. The p50 increased between 0 and 4 hours for both WT and MT samples. No difference was apparent in p50 values between the two thawing methods.

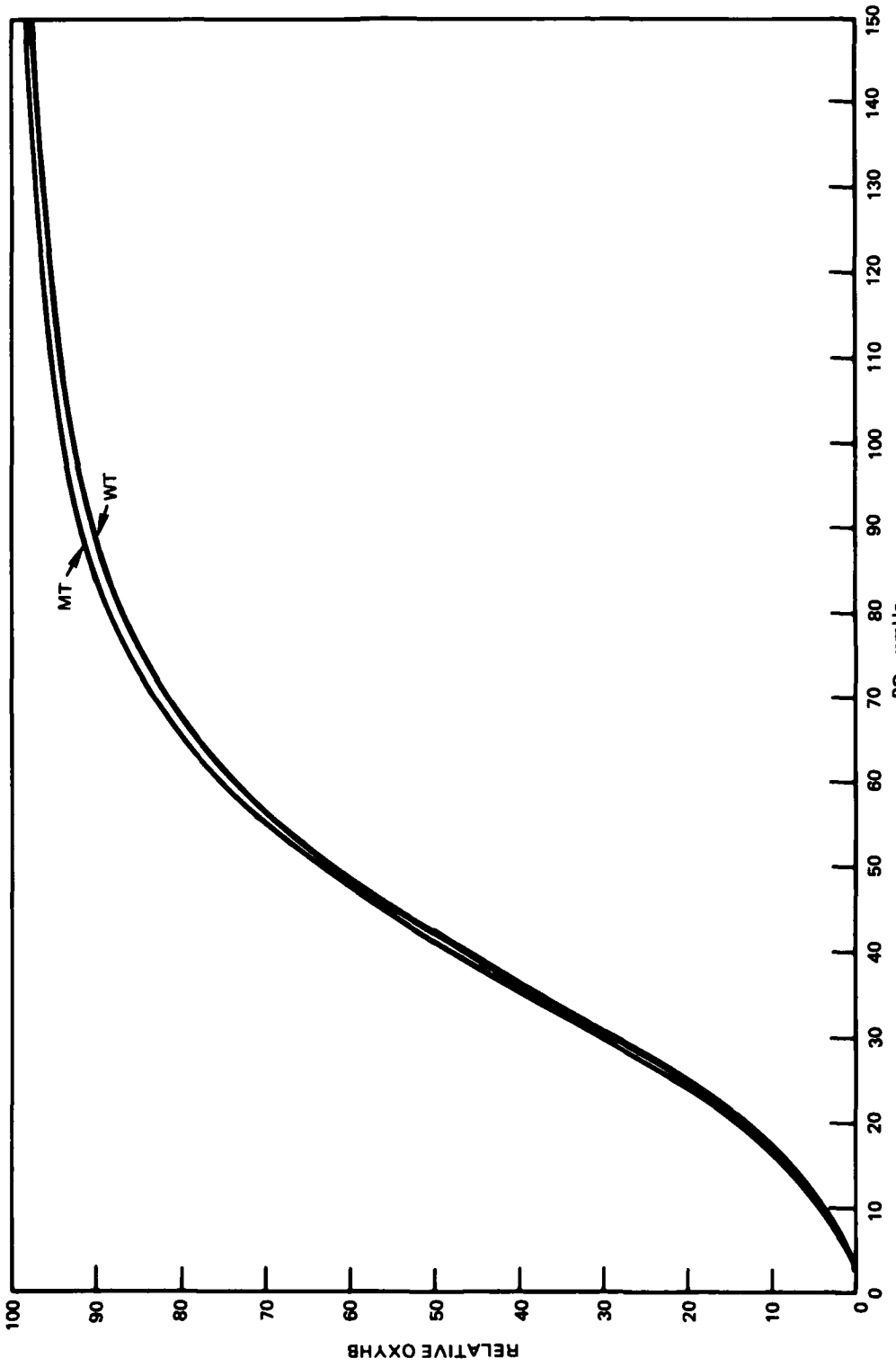


Figure 4. Oxygen dissociation curves of WT- and MT-RBCs at 4 hours post-wash.

Table 7. O_2 partial pressure (p50 in mmHg) necessary for 50% saturation of WT-and MT-RBC samples at hour 0 and hour 4 post-wash.

	UNIT 22			UNIT 23	
	WT	MT		WT	MT
8/7/80			8/14/80		
Hour 0	28.0	26.0	0	32.0	29.5
4	39.5	38.0	4	41.5	41.0
8/13/80			8/20/80		
Hour 0	28.0	30.0	0	42.0	42.0
4	33.5	32.5	4	43.0	43.5

Contamination studies were conducted on samples from Units 19 through 23 immediately post-thaw, at zero and at 6 hours post-wash. Cultures with five or more bacterial colonies per 0.5 ml of sample were considered contaminated. The data from any sample producing more than five colonies was discarded. No bacterial colonies were detected immediately post-thaw or at 0 hour. No more than two colonies per 0.5 ml of RBC sample were observed at 6 hours post-wash. one sample from Unit 23 produced 9 colonies at 6 hours, invalidating the data from this sample, and causing the data to be rejected from analysis.

Statistical Comparison

A comparison of microwave versus water bath thawed RBCs by means of a two way ANOVA test is shown in Table 8. Interactive effect (dependency of a difference observed between the two thawing treatments on individual units) is indicated by a '+'. Significant differences in thaw treatment (P 0.05) are identified in the table by an asterisk following the F ratio.

Table 8. Two way ANOVA F ratio statistics for morphologic and metabolic characteristics.

		%R	%H	Hb_t%	Hb_s%	Hct	Hb_c%	RBC#	MCV	MCHC	MCH	Glu	Glu (dc/dt)	Lac	Lac (dc/dt)	Pyr	ATP	GSH	DPG	pH
POST THAW	n	101	101	101	101	101				101										
	F	6.52*	6.43*	1.26	3.18	2.34	—	—	—	3.27	—	—	—	—	—	—	—	—	—	—
0 HOUR	n	96	96	96	96	96	96	92	92	96	92	95	90	84	84	40	95	60	96	90
	F	0.15	0.04	0.21	0.11	0.41	0.34	0.63	0.20	0.47	0.03	0.67	0.01	1.08	0.69	0.06	0.23	0.59	0.88	0.01
2 HOUR	n		96	96	96	96	96	84	84	96	84	96	86	84	82	40	94	60	96	92
	F	—	0.01	0.00	0.55	0.08	0.00	0.16	0.45	2.81	0.01	0.04	0.01	0.10	0.00	0.03	0.00	0.39	0.19	0.19
4 HOUR	n		94	94	94	94	94	82	82	94	82	94	88	82	81	40	92	60	94	90
	F	—	0.32	0.03	0.29	0.52	0.02	0.41	0.66	2.09	0.39	0.75	0.63	0.08	1.58	0.21	0.99	+	0.21	0.00
6 HOUR	n		94	94	94	94	94	82	82	94	82	92	91	82	82	40	90	57	94	80
	F	—	0.07	0.38	0.74	0.37	0.26	1.59	0.07	0.13	0.38	0.72	0.08	0.43	2.71	0.00	0.41	3.39	0.01	0.07

— No value

*Difference is significant at P=0.05.
+Interaction is significant at P=0.05.

DISCUSSION

STATISTICAL ANALYSIS

Two significant differences ($P \leq 0.05$) between MT- and WT-RBCs were observed using the two way ANOVA analysis (Table 8). The average freeze-thaw %R for MT-RBCs (97.3%) was significantly higher than the average freeze-thaw recovery for WT-RBCs (96.6%). In support of this difference, a significant difference in %H was also observed between MT-RBCs (3.8%) and WT-RBCs (4.4%) immediately post-thaw. No other measured parameters immediately after thaw or during the 6 hour incubation period showed significant differences.

The observed significant differences indicate that microwave thawing is less damaging to RBCs than water bath thawing. One possible reason for the increased cell survival may be faster thawing times: the small test tube RBC samples required approximately 90 seconds of microwave heating versus 5 minutes of water bath heating. Theoretically, fast thawing has the same advantages as fast freezing, as discussed in "BACKGROUND." Specifically, fast passage through phase change should minimize both the recrystallization of water into larger ice crystals and the duration of protein exposure to high salt concentrations. Unfortunately, fast microwave thawing of full units of RBCs is not as easily achieved as for the test tube samples. Because of the greater chance for localized overheating in large samples, microwave energy must be carefully, and therefore slowly, applied. The observed improvement in cellular recovery of microwave over water bath thawed test tube samples will probably not be extended to full unit samples.

In a previous analysis using a slightly smaller sample size, Campbell and Drewe (1981) reported significant differences in the mean glucose concentration at 6 hours post-wash, and in the rate of lactate increases between 4 and 6 hours, and between 0 and 6 hours. This previous study utilized a two sample comparison of means test (z statistic) in which all data from the individual blood units were pooled. By combining all data and testing for differences between microwave and water bath thaw treatments, the variability between the blood units was not considered. The two way ANOVA test used in this report takes into account variability between samples while testing for differences between the thaw methods. The ANOVA design is therefore considered a more reliable test for thawing differences when variations between units are present, as was the case in this study.

A single instance of a significant ($P<0.05$) interaction between the thawing method and RBC units was observed for the 4 hour post-wash GSH concentration. This instance suggests that microwave thawing affected individual blood units differently with respect to GSH concentration at 4 hours post-wash. A possible cause of the detected interaction may have been the wide variation in GSH concentration between outdated-rejuvenated and fresh samples. However, the significant F statistic for interaction persisted when outdated-rejuvenated samples were tested alone. A possible explanation for the interaction might be microwave influence on unit specific enzymes or biochemicals associated with GSH production or degradation, or on the driving force of GSH reactions. For example, a unit specific microwave influence on the concentration of intracellular oxidizing agents would affect each unit's GSH production differently. However, since no significant interaction was observed in GSH

concentration at 0, 2, and 6 hours, and since no other supporting interactions were observed in other measured biochemicals, the observed interaction at 4 hours is probably in error. Because of the calculated interaction significance, the F statistic for thawing method differences could not be calculated. The difference between thaw methods for GSH at 4 hour post-wash, however, is assumed insignificant.

TIME STUDIES

The major question addressed by this research, does microwave thawing affect RBC biochemistry, has been answered in the above section. Information regarding changes in post-thaw RBC biochemistry over time was also obtained from the experimental data, and is discussed in this section. For this time analysis, MT and WT samples were combined. Data from fresh-frozen samples was separated from the data of rejuvenated-frozen samples to observe the effect of rejuvenation on RBC metabolism.

Morphologic Parameters Over Time

Total Hb, Hct, MCV, MCH, and MCHC remained constant during the zero to 6 hour post-thaw testing period. As expected, Hb_s % and %H both progressively increased over the 6 hours of study. The MCV values remained lower than normal and MCHC values remained higher than normal, due to the slight hyperosmolality of the suspending medium.

Metabolic Parameters Over Time

Measuring the metabolic activity of RBCs aids in the prediction of in vivo survival and function. Metabolic activity was estimated by the cell's ability to produce ATP, 2,3-DPG, and GSH, and by the decrease in glucose and increase in lactate concentration over time.

Adenosine Triphosphate. Four moles of ATP are produced for each mole of glucose metabolized by the RBC. Since two moles of ATP are used in the glycolytic reaction, a net production of two moles of ATP per mole of glucose occurs. This ATP provides the cell with the energy needed to operate the Na^+/K^+ pump, to maintain normal intracellular cation concentrations, and to keep the RBC membrane intact (Rapaport, 1971).

The average post-thaw (MT and WT data combined) values of ATP for fresh and rejuvenated RBCs remained constant over 6 hours post-wash (see Figure 5), indicating adequate maintenance of zero-hour ATP concentrations. Rejuvenated RBC values were greater than fresh RBC values, demonstrating the advantage of the pre-freeze rejuvenation process. Valeri and Zaroulis (1972) reported a direct correlation of pre-transfusion ATP levels to post-transfusion survival of RBCs. The rejuvenated cells then, because of their initially high ATP concentration, and their ability to maintain this concentration (at least over short periods of time), would be clinically more effective in transfusion therapy.

2,3-Diphosphoglycerate. One Hb molecule, composed of four peptide chains (two α chains and two β chains) carries four molecules of oxygen. As Hb begins to give up oxygen, the chains move apart slightly, leaving a gap into

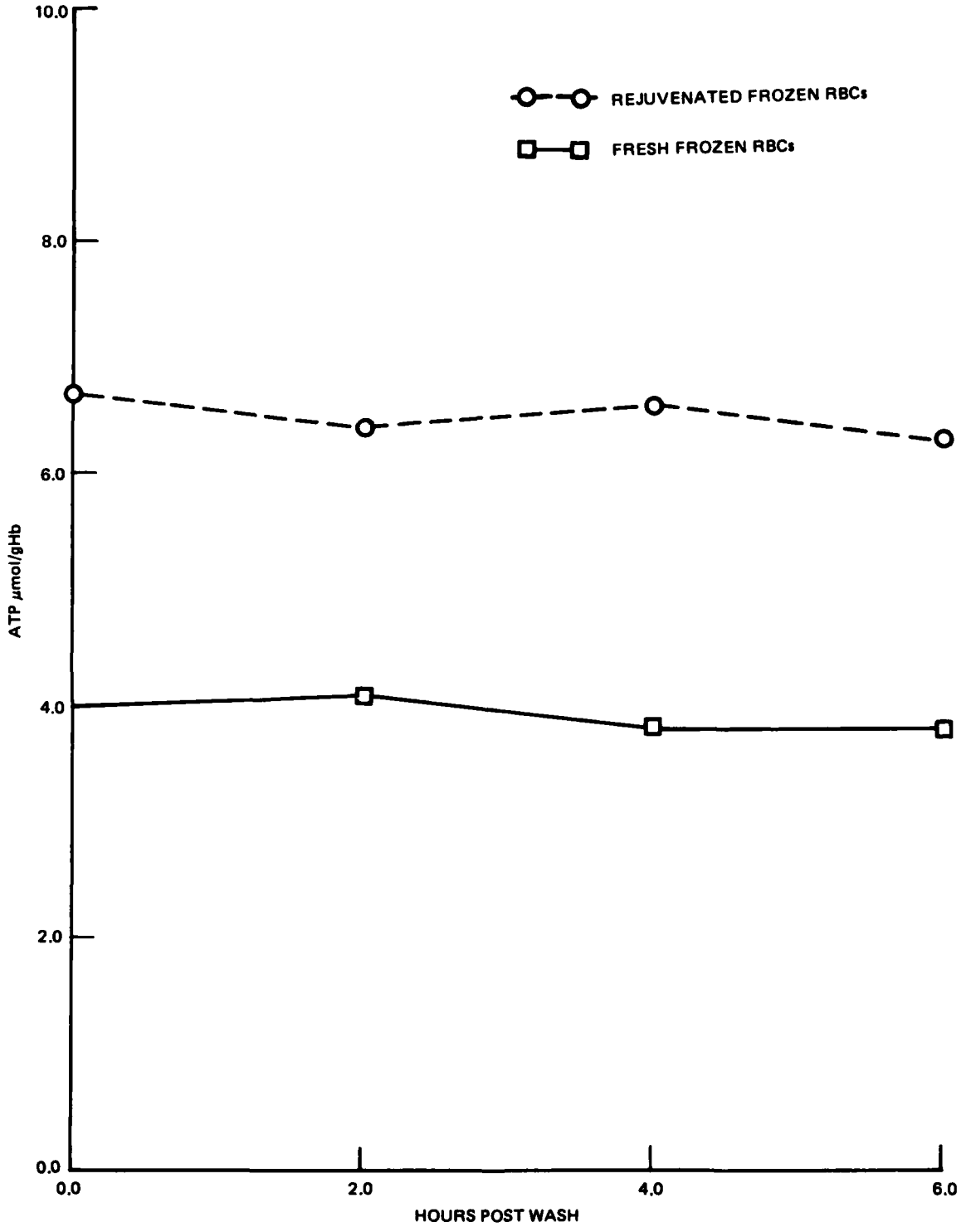


Figure 5. Changes in ATP concentration in RBCs over the 6 hours post-wash.

which a 2,3-DPG molecule will enter and bind (Rapaport, 1971). The reaction between Hb and 2,3-DPG may be considered:

$$HbO_2 + 2,3\text{-DPG} \rightarrow Hb\text{-}2,3\text{-DPG} + O_2 \text{ (Ganog, 1977)}$$

Thus, 2,3-DPG aids in releasing oxygen from Hb.

Figure 6 illustrates the effect of 2,3-DPG on the oxygen dissociation curve for whole blood. DPG depleted blood is represented by Curve 1, normal DPG levels by Curve 2, and elevated DPG levels by Curve 3. The difference Δa is the increased oxygen release from RBCs with elevated DPG levels over RBCs with normal DPG levels at a partial pressure of oxygen of 40 mm Hg. Increased DPG levels then decrease the oxygen affinity for Hb, shifting the curve to the right. The difference (Δb) is the decreased oxygen release of DPG depleted cells to tissues with 40 mm Hg oxygen. Low DPG levels then increase oxygen affinity, and shift the curve to the left.

The oxygen transport function of RBCs is especially critical during the first 4 to 8 hours post-transfusion (Valeri et al., 1977). Several hours are required to replenish DPG levels to normal in DPG depleted cells in vivo (Rapaport, 1971). Therefore, RBCs with low DPG levels (and cells with decreased ability to produce DPG) should not be transfused in hemorrhagic or septic shock patients, anemic patients with myocardial or cerebrovascular insufficiency, nor patients subjected to extracorporeal circulation during cardiac surgery (Valeri, 1976).

Figure 7 shows the decrease in DPG concentration over time in both fresh and outdated-rejuvenated RBCs. As can be seen, the rejuvenated cells again have elevated concentrations due to the rejuvenation process. The decrease in DPG over time is probably due to enzyme inhibition at pH values less than 7.4 (Wintch and James, 1977). As can be seen in Figure 8, the pH also decreased over time, passing the 7.4 mark between zero and 2 hours post-wash.

Reduced Glutathione. About 10% of the RBC's glucose is used in the hexose monophosphate shunt to produce reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is needed to reduce glutathione to GSH (Rapaport, 1971). Heinz bodies (precipitations in the Hb molecule due to te formation of sulfhydryl bridges between the chains) are formed when GSH concentrations are low (Heimpel, 1970). These Heinz bodies increase the rigidity of the cell, causing it to be removed from circulation by the spleen. Reduced glutathione also protects the Hb from oxidative denaturation to metHb by hydrogen peroxide (Rapaport, 1971; Blum, 1970).

GSH levels remained constant throughout the 6 hours of study (Figure 9). Again, outdated-rejuvenated cells showed higher GSH values due to the rejuvenation process. The relatively constant values over time indicate that the cells were not under oxidative stress which would have decreased the stockpiled GSH in the outdated-rejuvenated cells. The GSH production apparently is not as influenced by pH as is DPG production.

Metabolic Rate. Almost all of the glucose in RBC metabolism is converted to lactate — one mole of glucose is converted to two moles of lactate (Turner, 1970). The ratio of the rate of increase of lactate to the rate of decrease

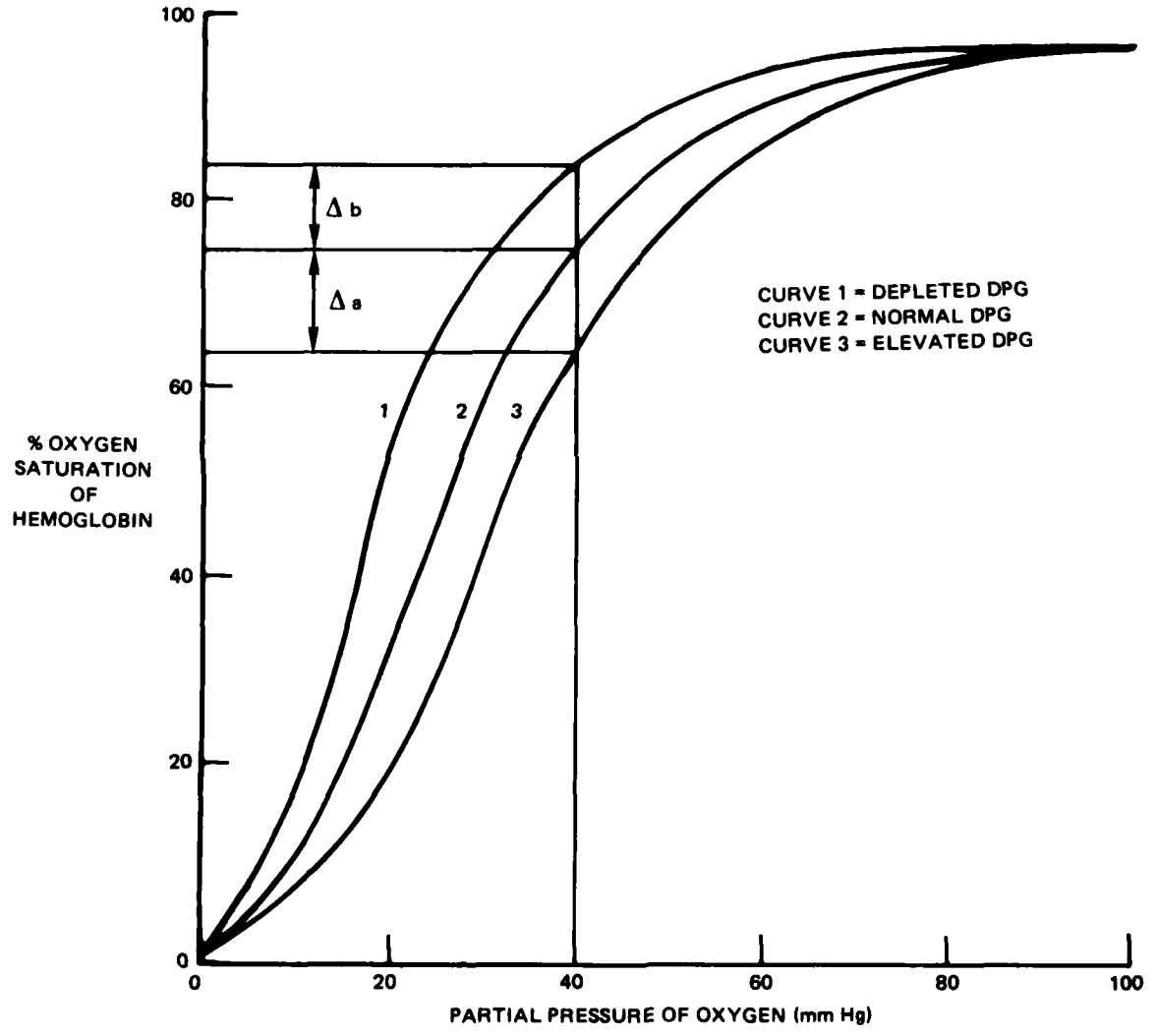


Figure 6. Hemoglobin-oxygen dissociation curve at three DPG levels.

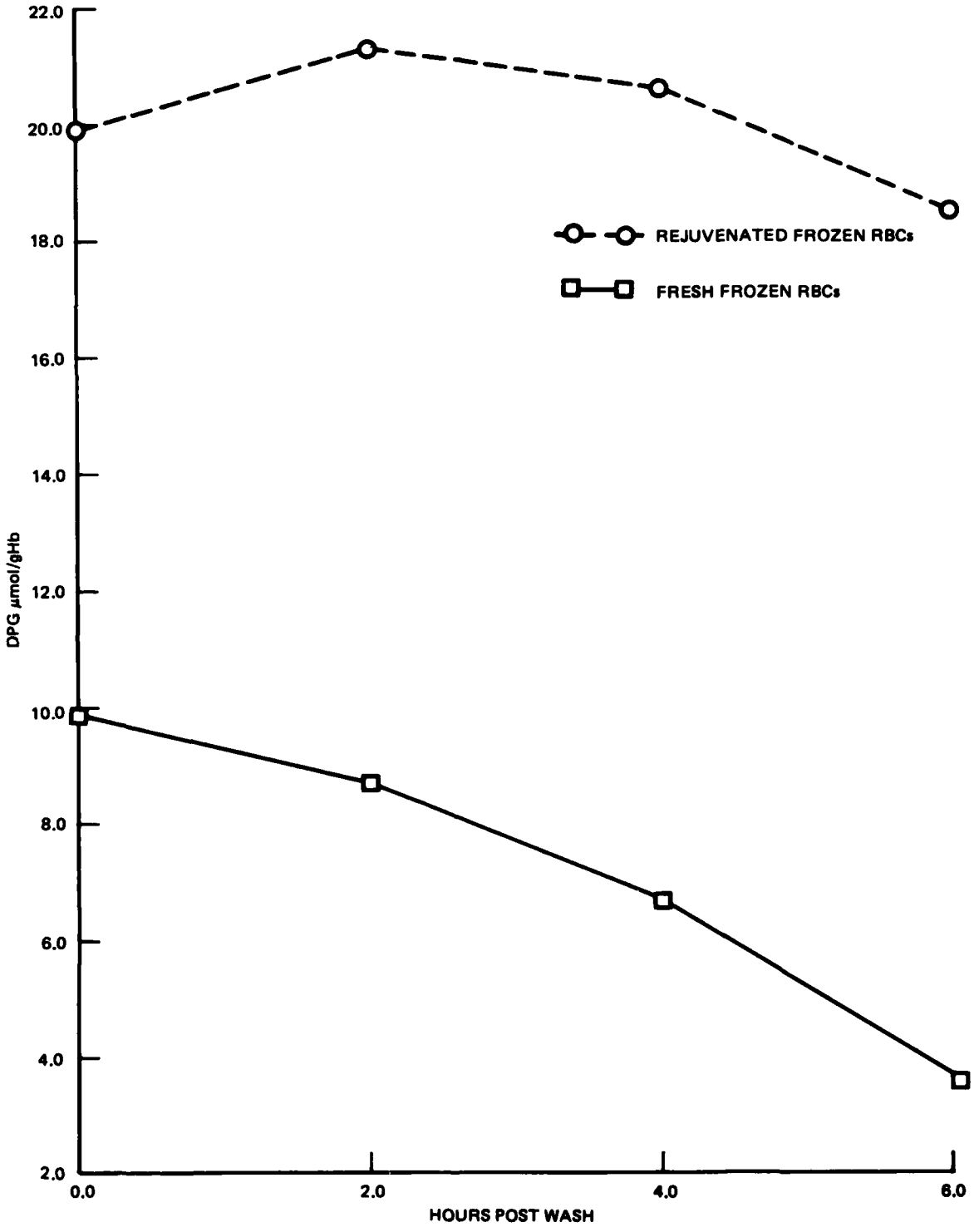


Figure 7. Changes in DPG concentration over the 6 hours post-wash.

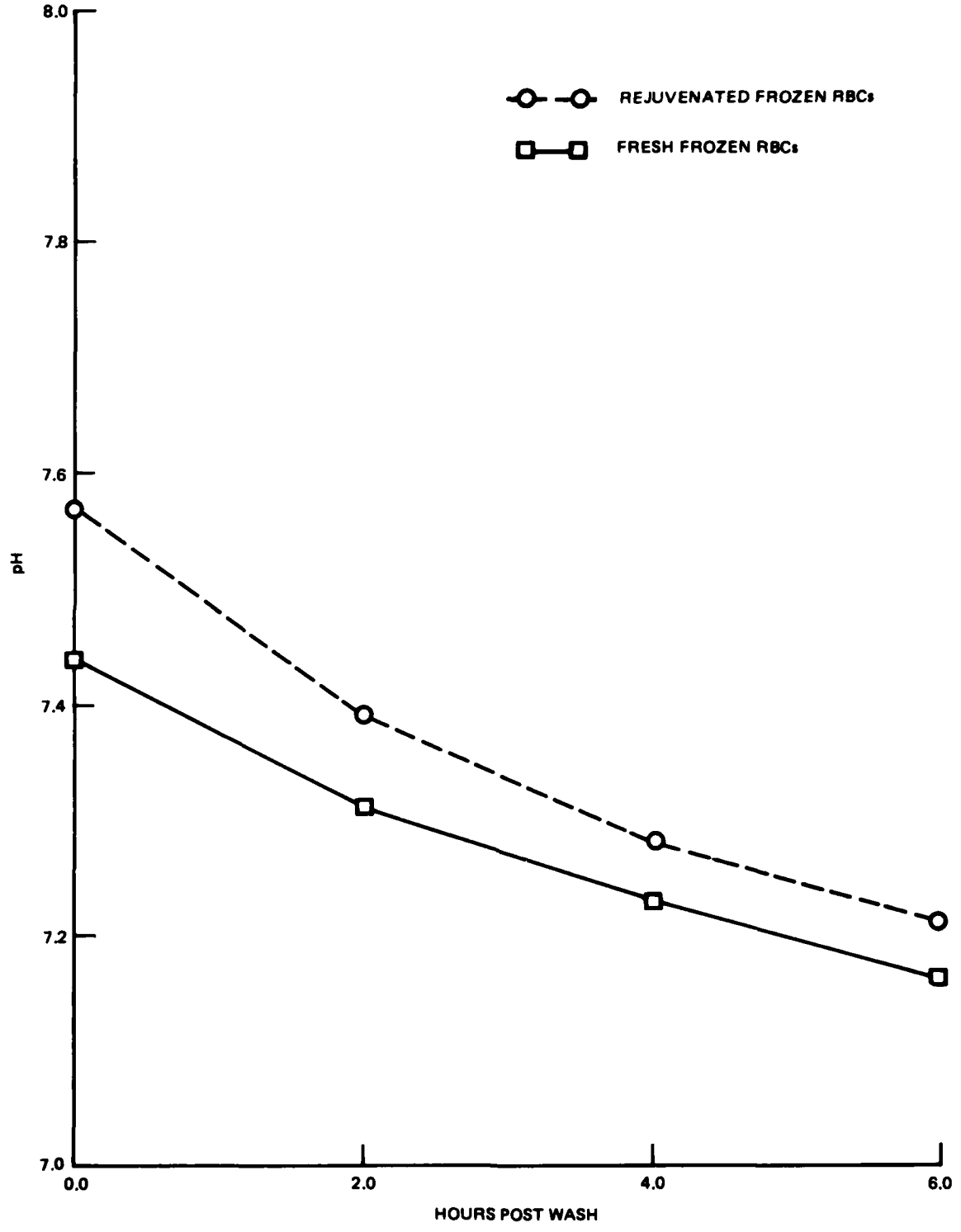


Figure 8. Changes in pH over the 6 hours post-wash.

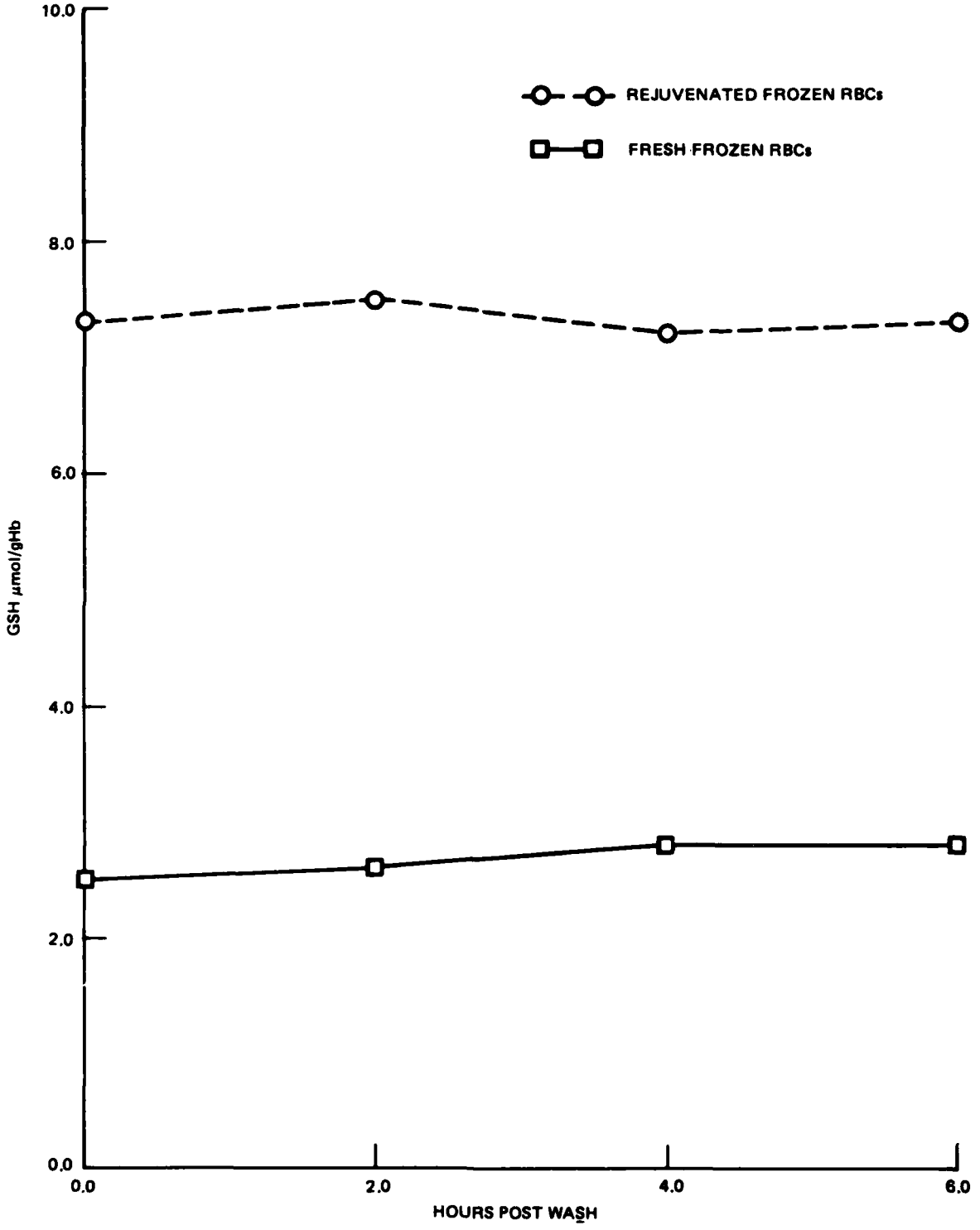


Figure 9. Changes in GSH concentration over the 6 hours post-wash.

of glucose should be two to one if all glucose is converted to lactate. However, some of the glucose is used in the hexose monophosphate shunt to keep glutathione reduced, so this ratio should actually be slightly less than two, depending on the cellular need for GSH.

Figures 10 and 11 represent the decreasing glucose concentration and increasing lactate concentration, respectively, over time. The lactate/glucose ratio is slightly less than two, as expected. Metabolic activity appears to have continued at a constant rate (note the slope of the lines) throughout the 6 hours of study, regardless of the lowering pH. No apparent difference was observed in metabolic rate between fresh and outdated-rejuvenated cells.

Preliminary studies (Campbell and Drewe, 1981) indicated a possible microwave-induced slowdown in metabolism (see STATISTICS). Therefore, samples in this study were analyzed for pyruvate to determine if the slowdown was occurring at the pyruvate-to-lactate step in the metabolic process. Using the ANOVA analysis and a larger sample size, no microwave-induced slowdown in metabolism was observed in either glucose decrease, lactate increase, or pyruvate production. Therefore, the initial purpose for studying pyruvate was no longer relevant. The changing pyruvate concentration over time, however, may be of interest and is shown in Figure 12. Pyruvate determinations were only made on units studied toward the end of the data collection period, and therefore were only made on outdated-rejuvenated cells.

Pyruvate concentrations increased dramatically towards the end of the 6 hour incubation period. This increase probably does not reflect an increase in pyruvate production, but rather a decrease in pyruvate degradation. The RBC's anaerobic metabolism converts glucose, through several intermediate biochemicals, to lactate. The last intermediate is pyruvate which is converted to lactate by the enzyme lactate dehydrogenase (LDH). Actually, this reaction is bidirectional and in an equilibrium controlled by both the LDH enzyme, and pyruvate and lactate concentrations.

$$\text{pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+ \qquad \text{(Lehninger, 1977)}$$

The large increase in pyruvate observed at 6 hours post-wash was most likely due to the large lactate concentrations at that time, and/or a pH effect on the LDH enzyme.

pH. The change in pH over the 6 hours of study is indicated in Figure 8. Since lactate concentrations increased over time, expectedly the pH decreased. The influence of the lowering pH has already been discussed with respect to the DPG decrease and the pyruvate increase. The pH level also has a strong influence on oxygen affinity for Hb, in what is termed the Bohr effect. As the pH falls, oxygen affinity decreases (right shift of the dissociation curve) due to the fact that deoxyhemoglobin binds H^+ more readily than does oxyhemoglobin. In tissues, the primary factor for the release of oxygen from the hemoglobin molecule is a low pO_2. The Bohr effect, however, can contribute an additional 1-2% unsaturation (Ganong, 1977).

Table 8 shows that p50 values increased between 0 and 4 hours post-wash, indicating a right shift of the dissociation curve. The decrease in DPG levels over time should have caused a left shift of the curve, therefore this

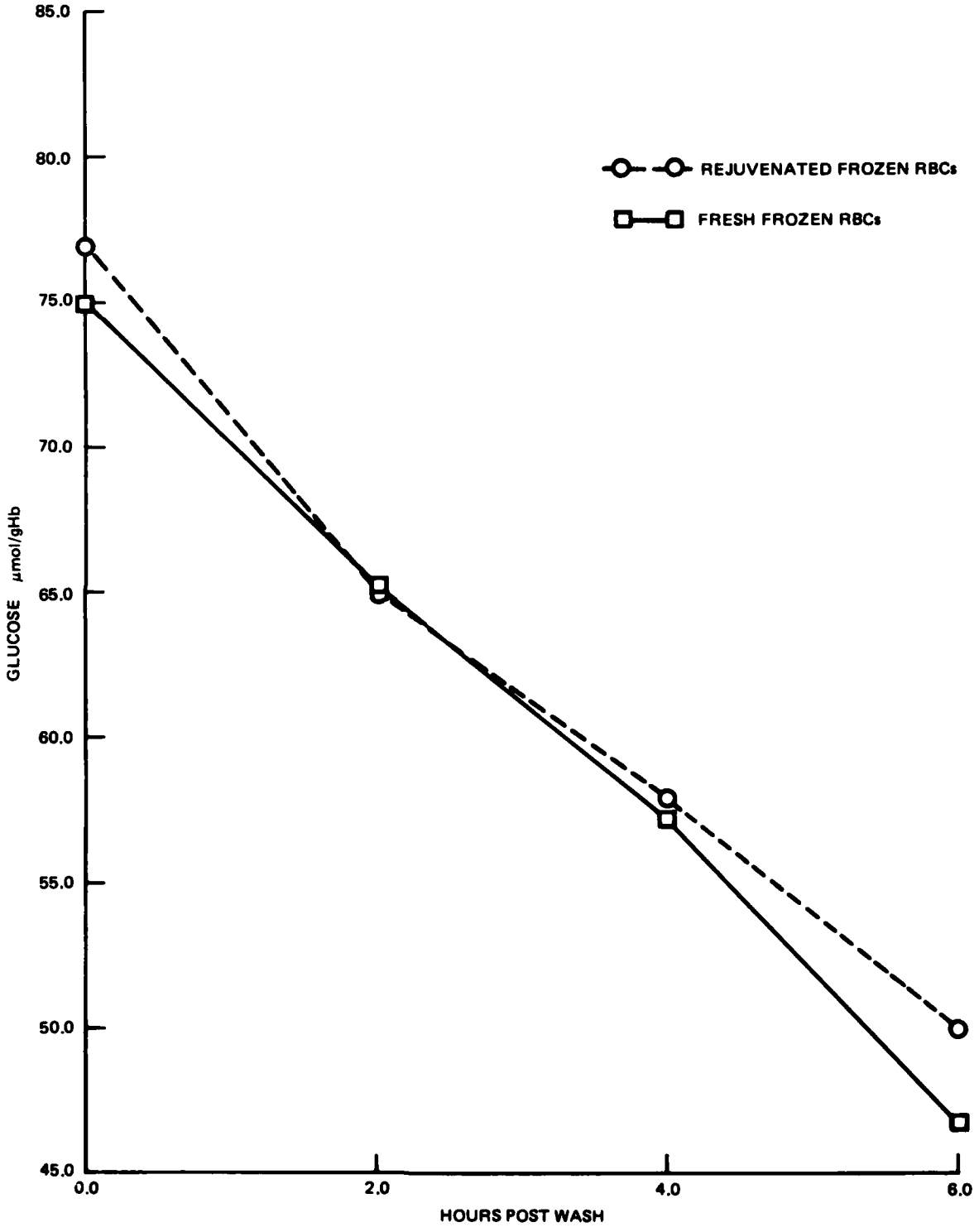


Figure 10. Changes in glucose concentration over the 6 hours post-wash.

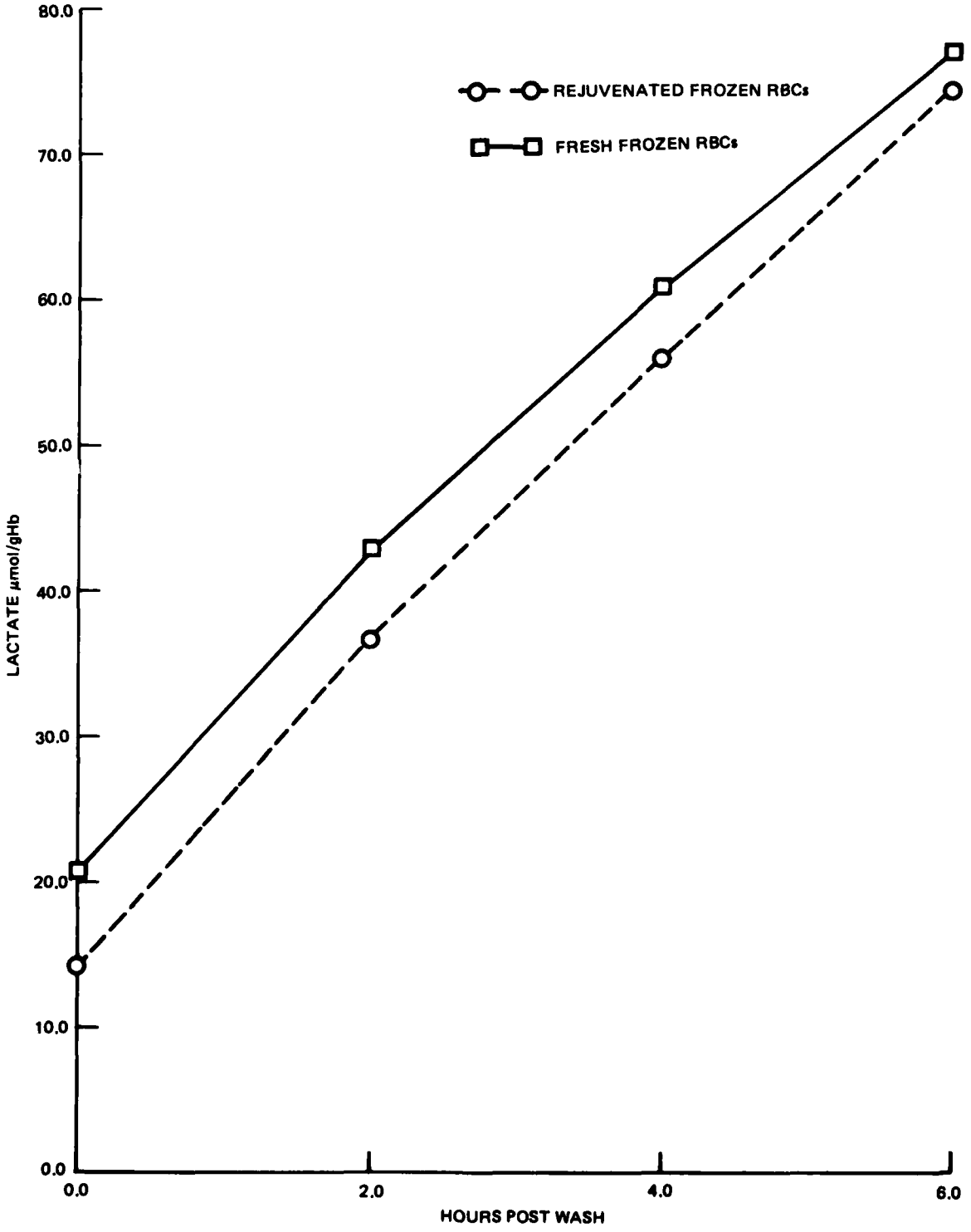


Figure 11. Changes in lactate concentration over the 6 hours post-wash.

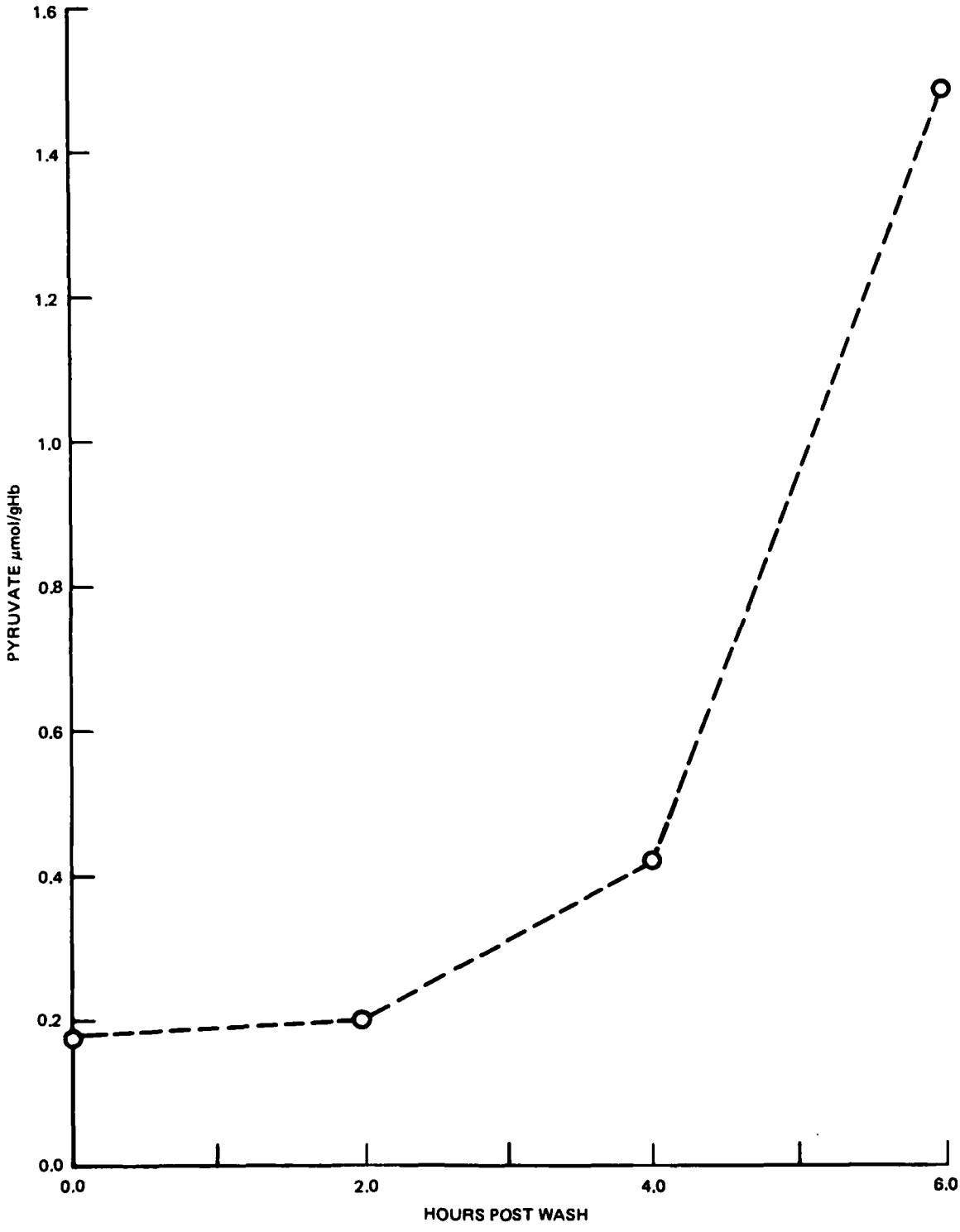


Figure 12. Changes in pyruvate concentration over the 6 hours post-wash.

apparent decrease in oxygen affinity must be another influence of the lowering pH.

CONCLUSION

Microwave thawing of RBCs does not appear to jeopardize the clinical use of frozen-thawed RBCs in transfusion therapy. The microwave thawed cells survived the freeze-thaw and wash procedures as well as (or better than) water bath thawed cells. The oxygen carrying function and the representative cellular metabolic processes remained intact. Future studies should be directed towards development and testing of a microwave thawing system for full units of RBCs.

REFERENCES

American Association of Blood Banks. 1977. Technical Manual. 7th ed. Philadelphia: J.B. Lippincott.

Beutler, E., O. Duron, and B.M. Kelly. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61:882-890.

Blum, K.U. 1970. "Biochemical aspects of erythrocyte aging in vivo and in vitro." In Modern Problems of Blood Preservation, edited by W. Spielmann and S. Seidl, 21-33. Stuttgart, Germany: Gustav Fischer Verlag.

Brown, B.A. 1976. Hematology: Principles and Procedures. 3rd ed. Philadelphia: Lea and Febiger.

Campbell, N.L. and J. Drewe. 1981. "Microwave thawing of frozen packed red blood cells." In IEEE MTT-S International Microwave Symposium Digest, edited by J.E. Raue, 479-481. 15-19 June 1981, Los Angeles.

Cleary, S. 1973. Uncertainties in the evaluation of the biological effects of microwave and radio frequency radiation. Health Physics 25:387-404.

Dalili, H. and J. Adriani. 1974. Effects of various blood warmers on the components of bank blood. Anesthesia and Analgesia 53(1):125-131.

Darrow, D.C. 1964. A Guide to Learning Fluid Therapy. Springfield: Charles C. Thomas.

Ganong, W.F. 1977. Review of Medical Physiology. Los Altos: Lange Medical Publications.

Georgia Institute of Technology. 1976. Electromagnetic Thawing of Frozen Granulocytes for Transfusion - Phase II, by E.C. Burdette. Atlanta: Georgia Institute of Technology.

Heimpel, H. 1970. "Factors determining red cell survival in normal and abnormal conditions." In Modern Problems of Blood Preservation, edited by W. Spielman and S. Seidl, 176-183. Stuttgart, Germany: Gustav Fischer Verlag.

Hurn, B.A. 1968. Storage of Blood. London: Academic Press.

Lehninger, A.L. 1977. Biochemistry. New York: Worth Publishers, Inc.

Lewis, G.P. 1965. Method using o-tolidine for the quantitative determination of hempoglobin in serum and urine. J. Clin. Path. 18:235-239.

Luyet, B.J. 1965. "Attempt at correlating some of the extant data on low-temperature preservation of various cells." In Cryobiology, edited by R.E. Stowell, 315-322. Washington: Fed. of Amer. Societies for Experimental Biology.

Mazur, P. 1965. "Causes of injury in frozen and thawed cells." In Cryobiology, edited by R.E. Stowell, 175-182. Washington: Fed. of Amer. Societies for Experimental Biology.

Moss, G.S., C.R. Valeri, and C.E. Brodine. 1968. Clinical experience with the use of frozen blood in combat casualities. New Eng. J. of Med. 278: 747-752.

Myhre, B.A., Y. Nakasako, and R. Schott. 1977a. A rapid method for determining the sterility of frozen-reconstituted blood. Transfusion 17(3):195-202.

Myhre, B.A., Y. Nakasako, and R. Schott. 1977b. Studies on 4°C stored frozen-reconstituted red blood cells: I. Bacterial growth. Transfusion 17(5):454-459.

Myhre, B.A., Y. Nakasako, and R. Schott. 1978. Studies on 4°C stored frozen-reconstituted red blood cells: II. Chemical and cytological changes during storage. Transfusion 28(1):29-37.

Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, and D.H. Bent, eds. 1975. Chapter 22.1.3. Statistical Package for the Social Sciences. 2d ed. McGraw Hill.

NRC Division of Medical Sciences. 1973. "The case for expanded frozen storage of red cells." In Preservation of Red Blood Cells, edited by H. Chaplin et al, 43-49. Washington: National Academy of Sciences.

Princeton Biomedix, Inc. 1976. Glutathione: An erythrocyte assay from Princeton Biomedix. Biomedix Bulletin 5004-20. Princeton: Princeton Biomedix, Inc.

Rajotte, R.V., J.B. Dossetor, W.A.G. Voss, and C.R. Stiller. 1974. Preservation Studies on canine Kidneys recovered from the deep frozen state by microwave thawing. Proc. of the IEEE 62(1):76-85.

Rapaport, S.I. 1971. Introduction to Hematology. Hagerstown, MD: Harper and Row.

Rapatz, G., and B. Luyet. 1973. The cryopreservation of blood by the method of two-step freezing. Biodynamica 11(239):169-179.

Sigma Chemical Company. 1974a. The ultraviolet enzymatic determination of 2, 3-diphosphyglyceric acid (2, 3-DPG) in blood at 340 nm. St. Louis: Sigma Tech. Bull. 35-UV.

Sigma Chemical Company. 1974b. The ultraviolet determination of ATP (adenosine-5'-triphosphate) in whole blood at 340 nm. St. Louis: Sigma Tech. Bull. 366-UV.

Sigma Chemical Company. 1976a. The enzymatic clorimetric determination of glucose in whole blood, plasma or serum at 425-475 nm. St. Louis: Sigma Tech. Bull. 510.

Sigma Chemical Company. 1976b. The quantitative determination of pyruvic acid and lactic acid in whole blood at 340 nm. St. Louis: Sigma Tech. Bull. 826-UV.

Sigma Chemical Company. 1976c. The quantitative calorimetric determination of total hemoglobin in whole blood at 530-550 nm. St. Louis: Sigma Tech. Bull. 525.

Simmons, A. 1968. Technical Hematology. Philadelphia: Lippincott.

Szymanski, I.O., and E.J. Carrington. 1977. Evaluation of a large-scale frozen blood program. Transfusion 17(5):431-437.

Travenol Laboratories, Inc., Fenwal Division. 1973. Quality Assurance in a Frozen Blood Program, by J.W. Garber and J.E. Voska. Deerfield: Travenol Laboratories, Inc.

Turner, A.R. 1970. Frozen blood. New York: Gordon and Breach.

U.S. Dept. of Health, Education and Welfare. 1970. Physical characteristics of microwave and other radio frequency radiation, by J.H. Vogelman. In Biological effects and health implications of microwave radiation, edited by S.F. Cleary. Rockville: Bureau of Radiological Health Publication No. BRH/DBE-70-2;1970:7-12.

U.S. Dept. of Health, Education and Welfare. 1976a. An aseptic fluid transfer system to maintain blood sterility, by R.M. Berkman, J.C. Arnett, E.L. Cleland, M.D. Wardle. In Frozen Red Cell Outdating, edited by P.B. Sherer. Washington: DHEW Pub. no. (NIH) 76-1004; 1976: 17-31.

U.S. Dept. of Health, Education and Welfare. 1976b. Bacteriological and biological quality of frozen red cells 72 hours following deglycerolization, by H.T. Meryman and R.A. Kahn. In Frozen Red Cell Outdating, edited by P.B. Sherer. Washington: DHEW Publication No. (NIH) 76-1004; 1976: 105-112.

U.S. Dept. of Health, Education and Welfare. 1976c. The growth of bacteria in frozen-reconstituted blood and methods for detection of contamination, by B.A. Myhre, Y. Nakasako, and R. Schott. In Frozen Red Cell Outdating, edited by P.B. Sherer. Washington: DHEW Publication No. (NIH) 76-1004; 1976: 81-91.

U.S. Dept of Health, Education and Welfare. 1976d. Sterile coupling device for deglycerolized red blood cells, by S.C. Roberts, D.T. Reuss, and G.L. Moore. In Frozen Red Cell Outdating, edited by P.B. Sherer. Washington: DHEW Publication No. (NIH) 76-1004; 1976: 43-51.

U.S. Department of Health, Education, and Welfare. 1979. Nosocomial Pseudomonas Cepacia infection. Morbidity and Mortality Weekly Report 28(25):289-290.

Valeri, C.R. 1974a, "Metabolic regeneration of depleted erythrocytes and their frozen storage." In The Human Red Cell in Vitro, edited by T.J. Greenwalt, and G.A. Jaimeson, 281-321. New York: Grune & Stratton, Inc.

Valeri, C.R. 1974b. Factors influencing the 24-hour posttransfusion survival and the oxygen transport function of previously frozen red cells preserved with 40 per cent W/V glycerol and frozen at -80 C. Transfusion 14(1):1-15.

Valeri, C.R. 1975. Blood components in the treatment of acute blood loss: use of freeze-preserved red cells, platelets, and plasma proteins. Anesthesia and Analgesia 54(1):1-14.

Valeri, C.R. 1976. "Cryobiology overview of red cell preservation: achievements and prospective." In Clinical Uses of Frozen-Thawed Red Blood Cells, edited by J.A. Gricp, 55-87. New York: Alan R. Liss, Inc.

Valeri, C.R., D.A. Valeri, J. Anastasi, C.G. Zaroulio, and J.J. Vecchione. 1977. Status report on red blood cell freezing: Biochemical modification and freeze-preservation in the original polyvinylchloride plastic collection bag. Paper presented at Advanced Component Seminar. 8 October, at the Haemonetics Research Institute. Available from the Naval Blood Research Laboratory, Boston, MA.

Valeri, C.R., D.A. Valeri, J. Anastasi, J.J. Vecchione, R.C. Dennis, C.A. Valeri, and C.P. Emerson. 1979. An improved red cell freezing system: Higher quality at lower cost. Status report for Office of Naval Research Contract No. NR-207-186. Available from the Naval Blood Research Laboratory, Boston, MA.

Valeri, C.R., and C.G. Zaroulis. 1972. Rejuvenation and freezing of outdated stored human red cells. New Eng. J. of Med. 287(26):1307-1312.

Williams, W.J., ed. 1972. Hematology. New York: McGraw-Hill.

Wintch, R., and P.M. James. 1977. Viability of cryopreserved red blood cells studied over 96 hr post-thaw. J. of Surg. Res. 23:88-95.

APPENDIX A

Morphologic Characteristics of

Water-Bath and Microwave Thawed Blood Samples

(Unit Breakdown)

		%R (%)								%H (%)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
POST THAW																	
WT	n	7	4	9	7	7	6	6	5	7	4	9	7	7	6	6	5
	$\bar{x}$	98.8	94.5	95.5	99.4	97.4	93.5	97.0	95.1	2.3	5.8	5.5	1.4	3.0	6.7	5.6	6.6
MT	n	7	4	9	7	6	6	6	5	7	4	9	7	6	6	6	5
	$\bar{x}$	98.8	96.1	96.8	98.7	97.6	95.8	97.3	96.3	2.3	3.7	4.1	2.1	2.9	4.7	5.3	5.5
Combined	n	14	8	18	14	13	12	12	10	14	8	18	14	13	12	12	10
	$\bar{x}$	98.8	95.3	96.1	99.1	97.5	94.6	97.2	95.7	2.3	4.8	4.8	1.8	3.0	5.7	5.4	6.0
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	64.5	69.7	84.4	90.3	86.6	71.3	86.9	82.3	0.9	0.8	0.9	1.4	0.7	1.1	0.6	0.8
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	65.2	75.7	84.0	90.8	86.8	64.6	85.3	80.5	1.0	0.6	0.8	1.9	0.6	1.1	0.6	0.8
Combined	n	12	8	18	14	12	12	12	8	12	8	18	14	12	12	12	8
	$\bar{x}$	64.8	72.7	84.2	90.6	86.7	67.9	86.1	81.4	1.0	0.7	0.9	1.7	0.7	1.1	0.6	0.8
2 HOUR																	
WT	n									6	4	9	7	6	6	6	4
	$\bar{x}$									1.8	0.8	0.9	1.3	0.8	1.2	0.7	0.9
MT	n									6	4	9	7	6	6	6	4
	$\bar{x}$									1.5	1.0	1.0	1.4	0.7	1.2	0.8	0.9
Combined	n									12	8	18	14	12	12	12	8
	$\bar{x}$									1.6	0.9	1.0	1.3	0.7	1.2	0.8	0.9
4 HOUR																	
WT	n									6	4	8	7	6	6	6	4
	$\bar{x}$									1.7	1.2	1.3	1.6	0.9	1.2	1.0	1.0
MT	n									6	4	8	7	6	6	6	4
	$\bar{x}$									1.4	1.2	1.2	1.4	0.8	1.2	0.9	0.9
Combined	n									12	8	16	14	12	12	12	8
	$\bar{x}$									1.6	1.2	1.2	1.5	0.9	1.2	0.9	0.9
6 HOUR																	
WT	n									6	4	8	7	6	6	6	4
	$\bar{x}$									1.9	1.7	1.5	1.8	1.2	1.4	1.3	1.3
MT	n									6	4	8	7	6	6	6	4
	$\bar{x}$									1.8	1.6	1.4	2.0	1.1	1.6	1.2	1.2
Combined	n									12	8	16	14	12	12	12	8
	$\bar{x}$									1.8	1.6	1.4	1.9	1.1	1.5	1.2	1.2

		Hb_t% (gm%)								Hb_s% (gm%)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
POST THAW																	
WT	n	7	4	9	7	7	6	6	5	7	4	9	7	7	6	6	5
	$\bar{x}$	20.9	15.9	16.3	20.1	21.5	23.1	23.1	21.5	1.5	1.8	1.7	0.8	2.0	4.3	3.7	3.9
MT	n	7	4	9	7	6	6	6	5	7	4	9	7	6	6	6	5
	$\bar{x}$	21.1	16.1	16.4	20.1	22.4	22.9	23.1	21.5	1.5	1.1	1.3	1.3	2.0	3.1	3.5	3.2
Combined	n	14	8	18	14	13	12	12	10	14	8	18	14	13	12	12	10
	$\bar{x}$	21.0	16.0	16.3	20.1	21.9	23.0	23.1	21.5	1.5	1.4	1.5	1.1	2.0	3.7	3.6	3.5
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	10.9	9.7	10.9	11.5	11.1	9.4	11.5	9.9	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	11.0	10.3	10.8	11.6	11.4	8.2	11.2	9.8	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1
Combined	n	12	8	18	14	12	12	12	8	12	8	18	14	12	12	12	8
	$\bar{x}$	11.0	10.0	10.9	11.5	11.3	8.8	11.3	9.9	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1
2 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	11.0	9.8	10.8	11.9	11.4	9.5	11.5	9.9	0.3	0.1	0.1	0.2	0.1	0.3	0.1	0.1
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	11.4	10.3	10.9	11.7	11.8	8.4	11.5	9.8	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1
Combined	n	12	8	18	14	12	12	12	8	12	8	18	14	12	12	12	8
	$\bar{x}$	11.2	10.0	10.9	11.8	11.6	9.0	11.5	9.9	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.1
4 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	11.0	9.9	11.1	11.6	11.2	9.5	11.6	10.0	0.2	0.2	0.2	0.3	0.2	0.1	0.1	0.2
MT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	11.0	10.3	11.1	11.9	11.7	8.3	11.5	9.8	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1
Combined	n	12	8	16	14	12	12	12	8	12	8	16	14	12	12	12	8
	$\bar{x}$	11.0	10.1	11.1	11.8	11.4	8.9	11.5	9.9	0.2	0.2	0.2	0.3	0.2	0.1	0.1	0.1
6 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	11.0	9.6	11.4	11.6	11.2	9.6	11.7	10.0	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2
MT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	11.1	10.5	11.2	11.5	11.4	8.2	11.5	9.8	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
Combined	n	12	8	16	14	12	12	12	8	12	8	16	14	12	12	12	8
	$\bar{x}$	11.0	10.1	11.3	11.6	11.3	8.9	11.6	9.9	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2

		Hct (%)								Hb_C % (gm%)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
POST THAW																	
WT	n	7	4	9	7	7	6	6	5								
	$\bar{x}$	66.9	47.6	47.9	66.3	67.6	64.7	65.7	62.7								
MT	n	7	4	9	7	6	6	6	5								
	$\bar{x}$	67.8	48.0	48.3	66.2	67.7	64.9	65.2	62.8								
Combined	n	14	8	18	14	13	12	12	10								
	$\bar{x}$	67.3	47.8	48.1	66.3	67.6	64.8	65.4	62.7								
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	30.6	25.3	28.2	32.5	28.9	22.3	27.2	27.0	10.8	9.7	10.8	11.3	11.1	9.4	11.4	9.8
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	30.6	26.6	27.8	32.4	30.0	19.3	26.8	26.1	10.9	10.2	10.7	11.4	11.2	8.2	11.1	9.7
Combined	n	12	8	18	14	12	12	12	8	12	8	18	14	12	12	12	8
	$\bar{x}$	30.6	25.9	28.0	32.5	29.4	20.8	27.0	26.6	10.9	10.0	10.8	11.3	11.1	8.8	11.2	9.8
2 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	30.3	25.3	27.8	32.6	29.3	22.8	27.8	27.3	10.9	9.7	10.6	11.7	11.1	9.4	11.4	9.8
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	4
	$\bar{x}$	31.2	26.9	28.0	32.5	30.5	19.8	27.1	26.3	11.2	10.2	10.8	11.5	11.6	8.3	11.4	9.7
Combined	n	12	8	18	14	12	12	12	8	12	8	18	14	12	12	12	S
	$\bar{x}$	30.8	26.1	27.9	32.6	29.9	21.3	27.5	26.8	11.0	9.9	10.7	11.6	11.3	8.8	11.4	9.8
4 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	30.4	25.3	28.5	32.5	29.3	22.7	28.2	27.4	10.8	9.8	11.0	11.5	11.1	9.3	11.5	9.9
MT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	30.3	27.1	28.1	32.5	29.9	19.8	27.2	26.7	10.8	10.2	11.0	11.7	11.6	8.2	11.3	9.7
Combined	n	12	8	16	14	12	12	12	8	12	8	16	14	12	12	12	8
	$\bar{x}$	30.3	26.2	28.3	32.5	29.6	21.2	27.7	27.0	10.8	10.0	11.0	11.6	11.3	8.7	11.4	9.8
6 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	30.5	25.3	28.6	32.6	29.2	22.9	27.9	27.5	10.8	9.5	11.2	11.3	11.1	9.5	11.5	9.9
MT	n	6	4	8	7	6	6	6	4	6	4	8	7	6	6	6	4
	$\bar{x}$	30.6	27.2	28.5	32.9	29.7	19.4	27.3	26.5	10.9	10.4	11.0	11.3	11.3	8.1	11.4	9.7
Combined	n	12	8	16	14	12	12	12	8	12	8	16	14	12	12	12	8
	$\bar{x}$	30.5	26.2	28.5	32.8	29.4	21.2	27.6	27.0	10.8	9.9	11.1	11.3	11.2	8.8	11.4	9.8

		RBC# ($\times 10^6/mm^3$)								MCV (μ^3)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
POST THAW																	
WT	n																
	$\bar{x}$																
MT	n																
	$\bar{x}$																
Combined	n																
	$\bar{x}$																
0 HOUR																	
WT	n	5	4	9	7	6	6	6	4	5	4	9	7	6	6	6	4
	$\bar{x}$	3.3	3.3	3.5	4.1	3.9	3.2	3.7	3.4	89.4	78.0	81.6	79.5	73.5	70.5	73.4	78.8
MT	n	3	4	9	7	6	6	6	4	3	4	9	7	6	6	6	4
	$\bar{x}$	3.0	3.4	3.5	4.1	4.0	2.8	3.7	3.3	91.8	78.2	79.9	79.0	75.4	68.3	73.3	78.7
Combined	n	8	8	18	14	12	12	12	8	8	8	18	14	12	12	12	8
	$\bar{x}$	3.2	3.3	3.5	4.1	4.0	3.0	3.7	3.4	90.3	78.1	80.7	79.3	74.5	69.4	73.4	78.8
2 HOUR																	
WT	n		4	9	7	6	6	6	4		4	9	7	6	6	6	4
	$\bar{x}$		3.3	3.5	4.2	3.9	3.2	3.8	3.4		78.0	79.3	78.3	75.0	71.9	74.4	81.2
MT	n		4	9	7	6	6	6	4		4	9	7	6	6	6	4
	$\bar{x}$		3.4	3.5	4.1	4.1	2.9	3.7	3.3		79.5	79.6	78.7	73.4	68.8	74.1	80.2
Combined	n		8	18	14	12	12	12	8		8	18	14	12	12	12	8
	$\bar{x}$		3.3	3.5	4.2	4.0	3.0	3.7	3.3		78.7	79.5	78.5	74.2	70.3	74.3	80.7
4 HOUR																	
WT	n		4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$		3.4	3.5	4.0	4.0	3.1	3.8	3.5		77.5	81.3	80.9	73.7	72.3	74.0	80.3
MT	n		4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$		3.4	3.5	4.2	4.1	2.8	3.7	3.4		78.7	82.6	78.6	73.8	70.8	73.5	79.1
Combined	n		8	16	14	12	12	12	8		8	16	14	12	12	12	8
	$\bar{x}$		3.4	3.5	4.1	4.0	3.0	3.8	3.4		78.1	81.9	79.7	73.8	71.6	73.8	79.7
6 HOUR																	
WT	n		4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$		3.3	3.5	4.1	4.0	3.2	3.9	3.4		78.0	80.8	79.0	73.6	72.3	72.6	80.4
MT	n		4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$		3.4	3.5	4.1	4.0	2.7	3.7	3.3		79.5	80.8	79.8	73.4	71.0	73.3	80.3
Combined	n		8	16	14	12	12	12	8		8	16	14	12	12	12	8
	$\bar{x}$		3.3	3.5	4.1	4.0	2.9	3.8	3.4		78.8	80.8	79.4	73.5	71.7	73.0	80.3

		MCHC (%)								MCH (µµg)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
POST THAW																	
WT	n	7	4	9	7	7	6	6	5								
	$\bar{x}$	30.5	31.5	32.1	29.9	30.7	33.4	33.3	32.0								
MT	n	7	4	9	7	6	6	6	5								
	$\bar{x}$	30.4	32.3	32.5	29.7	32.2	33.6	33.6	32.3								
Combined	n	14	8	18	14	13	12	12	10								
	$\bar{x}$	30.4	31.9	32.3	29.8	31.4	33.5	33.4	32.1								
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	5	4	9	7	6	6	6	4
	$\bar{x}$	35.3	38.2	38.4	34.6	38.4	42.0	41.9	36.7	31.7	29.8	31.4	27.6	28.1	29.6	30.8	28.8
MT	n	6	4	9	7	6	6	6	4	3	4	9	7	6	6	6	4
	$\bar{x}$	35.9	38.5	38.5	35.1	37.6	42.2	41.6	37.4	32.7	30.1	30.7	27.8	28.3	28.8	30.5	29.4
Combined	n	12	8	18	14	12	12	12	8	8	8	18	14	12	12	12	8
	$\bar{x}$	35.6	38.4	38.5	34.9	38.0	42.1	41.8	37.1	32.1	30.0	31.1	27.7	28.2	29.2	30.7	29.1
2 HOUR																	
WT	n	6	4	9	7	6	6	6	4		4	9	7	6	6	6	4
	$\bar{x}$	35.8	38.3	38.3	35.9	37.9	41.4	40.9	35.1		29.8	30.4	28.1	28.3	29.7	30.4	29.5
MT	n	6	4	9	7	6	6	6	4		4	9	7	6	6	6	4
	$\bar{x}$	36.1	37.9	38.7	35.4	38.0	42.1	42.0	37.0		30.2	30.8	27.9	27.9	29.0	31.1	29.6
Combined	n	12	8	18	14	12	12	12	8		8	18	14	12	12	12	8
	$\bar{x}$	35.9	38.1	38.5	35.7	37.9	41.7	41.5	36.0		30.0	30.6	28.0	28.1	29.3	30.8	29.5
4 HOUR																	
WT	n	6	4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$	35.5	38.6	38.4	35.2	37.7	41.1	40.8	35.9		29.9	31.2	28.4	27.8	29.7	30.2	28.8
MT	n	6	4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$	35.8	37.7	38.6	36.1	38.6	41.2	41.8	36.6		29.7	31.8	28.3	28.5	29.2	30.7	28.8
Combined	n	12	8	16	14	12	12	12	8		8	16	14	12	12	12	8
	$\bar{x}$	35.6	38.2	38.5	35.6	38.2	41.2	41.3	36.2		29.8	31.5	28.4	28.2	29.5	30.5	28.8
6 HOUR																	
WT	n	6	4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$	35.3	37.5	39.1	34.8	37.9	41.3	41.6	36.0		29.3	31.6	27.5	27.9	29.8	29.9	28.9
MT	n	6	4	8	7	6	6	6	4		4	8	7	6	6	6	4
	$\bar{x}$	35.7	38.1	38.8	34.3	38.1	41.7	41.7	36.6		30.3	31.4	27.3	28.0	29.6	30.6	29.3
Combined	n	12	8	16	14	12	12	12	8		8	16	14	12	12	12	8
	$\bar{x}$	35.5	37.8	39.0	34.5	38.0	41.5	41.6	36.3		29.8	31.5	27.4	27.9	29.7	30.2	29.1

APPENDIX B

Metabolic Characteristics of

Water Bath and Microwave Thawed Blood Samples

(Unit Breakdown)

		Glucose (μmol/gHb)								Glucose Rate of Decrease (μmol/gHb • min)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
0 HOUR																	
WT	n	6	4	8	7	6	6	6	4	5	4	8	6	6	5	6	3
	$\bar{x}$	95.5	88.8	66.0	53.2	74.6	91.0	63.2	86.3	8.0	11.4	8.3	9.2	11.0	12.0	11.6	8.7
MT	n	6	4	9	7	6	6	6	4	6	4	9	7	6	6	6	3
	$\bar{x}$	100.1	82.3	67.0	57.7	73.8	103.7	63.8	83.6	9.5	8.1	9.0	10.4	12.5	12.8	11.5	3.3
Combined	n	12	8	17	14	12	12	12	8	11	8	17	13	12	11	12	6
	$\bar{x}$	97.8	85.5	66.5	55.5	74.2	97.4	63.5	84.9	8.9	9.8	8.6	9.8	11.7	12.4	11.5	6.0
2 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	8	7	6	5	5	4
	$\bar{x}$	89.2	75.1	56.2	45.3	61.4	79.1	49.3	78.5	7.6	8.1	9.2	9.1	8.6	7.0	8.0	4.5
MT	n	6	4	9	7	6	6	6	4	5	4	8	6	6	4	5	3
	$\bar{x}$	88.7	72.6	54.4	44.4	59.0	88.4	50.1	81.8	8.7	9.3	9.2	7.5	8.3	8.6	7.7	4.9
Combined	n	12	8	18	14	12	12	12	8	11	8	16	13	12	9	10	7
	$\bar{x}$	88.9	73.8	55.3	44.9	60.2	83.7	49.7	80.1	8.1	8.7	9.2	8.3	8.4	7.7	7.9	4.7
4 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	2	8	6	5	6	6	3
	$\bar{x}$	80.1	65.3	45.3	37.4	51.0	72.6	43.2	73.1	11.9	9.4	7.7	9.7	5.5	8.1	5.1	5.3
MT	n	6	4	8	7	6	6	6	4	6	3	8	7	6	6	6	4
	$\bar{x}$	83.7	61.3	46.6	34.4	49.0	87.0	44.7	79.5	9.6	5.9	8.4	6.5	3.4	9.9	5.6	7.6
Combined	n	12	8	16	14	12	12	12	8	12	5	16	13	11	12	12	7
	$\bar{x}$	81.9	63.3	45.9	35.9	50.0	79.8	44.0	76.3	10.7	7.3	8.0	8.0	4.3	9.0	5.3	6.6
6 HOUR																	
WT	n	6	4	8	7	6	6	6	3	6	4	7	7	6	6	6	3
	$\bar{x}$	66.8	60.4	36.1	27.7	45.8	62.8	37.1	70.5	8.0	7.9	8.3	7.1	8.0	7.8	7.3	6.0
MT	n	6	4	8	7	6	6	6	3	6	4	8	7	6	6	6	3
	$\bar{x}$	72.3	56.5	36.4	26.7	45.0	75.2	38.0	72.4	7.8	7.2	8.8	8.6	8.1	7.9	7.2	4.1
Combined	n	12	8	16	14	12	12	12	6	12	8	15	14	12	12	12	6
	$\bar{x}$	69.5	58.4	36.2	27.2	45.4	69.0	37.6	71.4	7.9	7.5	8.5	7.9	8.0	7.9	7.2	5.0

		Lactate (µmol/gHb)								Lactate Rate of Increase (µmol/gHb • min)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
0 HOUR																	
WT	n	6	4	8	7	6	6	4	1	6	4	8	7	6	6	4	1
	$\bar{x}$	13.9	19.5	20.0	24.4	9.7	7.5	8.4	16.2	17.9	18.8	19.8	20.2	19.6	16.0	19.6	19.3
MT	n	6	4	8	7	6	6	4	1	6	4	8	7	6	6	4	1
	$\bar{x}$	15.4	22.0	21.5	27.4	10.8	9.0	9.1	15.5	16.4	17.7	21.2	18.2	18.6	15.3	20.1	19.7
Combined	n	12	8	16	14	12	12	8	2	12	8	16	14	12	12	8	2
	$\bar{x}$	14.6	20.7	20.7	25.9	10.2	8.3	8.8	15.9	17.2	18.3	20.5	19.2	19.1	15.7	19.9	19.5
2 HOUR																	
WT	n	6	4	8	7	6	6	4	1	6	4	7	7	6	6	4	1
	$\bar{x}$	35.4	42.1	47.0	49.2	33.2	26.8	32.0	39.3	14.8	19.0	14.8	14.7	17.8	14.7	18.3	15.0
MT	n	6	4	8	7	6	6	4	1	6	4	7	7	6	6	4	1
	$\bar{x}$	35.1	43.2	43.7	48.7	33.1	27.4	33.2	39.1	15.5	16.9	14.9	15.5	17.1	16.1	16.8	13.3
Combined	n	12	8	16	14	12	12	8	2	12	8	14	14	12	12	8	2
	$\bar{x}$	35.2	42.7	45.3	49.0	33.2	27.1	32.6	39.2	15.1	17.9	14.8	15.1	17.4	15.4	17.6	14.2
4 HOUR																	
WT	n	6	4	7	7	6	6	4	1	6	4	7	7	6	6	4	1
	$\bar{x}$	53.1	64.9	61.3	67.3	54.7	44.5	53.9	57.7	12.7	17.3	19.8	14.5	13.9	16.2	14.1	11.8
MT	n	6	4	7	7	6	6	4	1	5	4	7	7	6	6	4	1
	$\bar{x}$	53.6	63.5	65.0	66.8	53.6	46.7	53.4	55.1	10.4	12.8	12.7	17.0	15.1	16.5	14.1	13.7
Combined	n	12	8	14	14	12	12	8	2	11	8	14	14	12	12	8	2
	$\bar{x}$	53.4	64.2	63.2	67.0	54.1	45.6	53.6	56.4	11.6	15.0	16.3	15.8	14.5	16.4	14.1	12.8
6 HOUR																	
WT	n	6	4	7	7	6	6	4	1	6	4	7	7	6	6	4	1
	$\bar{x}$	68.3	85.6	85.1	84.8	71.3	63.9	70.8	71.5	15.1	18.3	17.9	16.8	17.1	15.6	17.3	15.4
MT	n	6	4	7	7	6	6	4	1	6	4	7	7	6	6	4	1
	$\bar{x}$	63.7	78.9	80.1	87.2	71.7	66.5	70.3	71.5	13.4	15.8	16.1	16.6	16.9	16.0	17.0	15.6
Combined	n	12	8	14	14	12	12	8	2	12	8	14	14	12	12	8	2
	$\bar{x}$	66.0	82.3	82.6	86.0	71.5	65.2	70.6	71.5	14.3	17.1	17.0	16.7	17.0	15.8	17.2	15.5

Pyruvate (μmol/gHb)

		15	17	18	19	20	21	22	23
0 HOUR									
WT	n					6	6	4	4
	$\bar{x}$					0.21	0.19	0.14	0.14
MT	n					6	6	4	4
	$\bar{x}$					0.16	0.18	0.18	0.21
Combined	n					12	12	8	8
	$\bar{x}$					0.18	0.18	0.16	0.18
2 HOUR									
WT	n					6	6	4	4
	$\bar{x}$					0.16	0.24	0.13	0.24
MT	n					6	6	4	4
	$\bar{x}$					0.15	0.33	0.10	0.19
Combined	n					12	12	8	8
	$\bar{x}$					0.15	0.28	0.12	0.21
4 HOUR									
WT	n					6	6	4	4
	$\bar{x}$					0.35	0.23	0.26	0.92
MT	n					6	6	4	4
	$\bar{x}$					0.43	0.20	0.33	0.94
Combined	n					12	12	8	8
	$\bar{x}$					0.39	0.21	0.29	0.93
6 HOUR									
WT	n					6	6	4	4
	$\bar{x}$					1.47	0.98	1.32	2.36
MT	n					6	6	4	4
	$\bar{x}$					2.04	0.53	1.33	2.20
Combined	n					12	12	8	8
	$\bar{x}$					1.76	0.76	1.33	2.28

		ATP (µmol/gHb)								GSH (µmol/gHb)							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	5			3	6	6	6	4
	$\bar{x}$	3.8	7.0	6.3	4.2	6.5	7.1	6.4	7.4	2.0			3.2	7.9	8.5	5.7	7.0
MT	n	6	4	8	7	6	6	6	4	5			3	6	6	6	4
	$\bar{x}$	3.7	6.7	6.6	4.2	6.6	7.2	6.5	7.5	2.2			3.1	8.1	8.7	5.6	6.9
Combined	n	12	8	17	14	12	12	12	8	10			6	12	12	12	8
	$\bar{x}$	3.8	6.8	6.4	4.2	6.5	7.2	6.5	7.4	2.1			3.2	8.0	8.6	5.6	7.0
2 HOUR																	
WT	n	6	4	9	7	5	6	6	4	5			3	6	6	6	4
	$\bar{x}$	4.0	6.7	5.9	4.3	6.3	6.8	6.3	7.1	2.3			3.3	8.6	8.7	5.4	7.7
MT	n	6	4	9	7	5	6	6	4	5			3	6	6	6	4
	$\bar{x}$	3.9	6.6	6.1	4.2	6.2	6.8	6.3	7.3	2.4			2.4	8.3	8.7	5.5	7.6
Combined	n	12	8	18	14	10	12	12	8	10			6	12	12	12	8
	$\bar{x}$	3.9	6.7	6.0	4.3	6.3	6.8	6.3	7.2	2.4			2.9	8.4	8.7	5.5	7.6
4 HOUR																	
WT	n	6	4	8	7	6	6	6	3	5			3	6	6	6	4
	$\bar{x}$	3.9	6.8	6.4	4.0	6.8	6.9	6.2	6.7	2.5			3.3	8.2	8.2	5.4	7.1
MT	n	6	4	8	7	6	6	6	3	5			3	6	6	6	4
	$\bar{x}$	3.7	6.8	6.1	3.7	6.4	7.3	6.3	6.6	2.5			3.3	7.6	8.6	5.4	7.3
Combined	n	12	8	16	14	12	12	12	6	10			6	12	12	12	8
	$\bar{x}$	3.8	6.8	6.2	3.9	6.6	7.1	6.2	6.6	2.5			3.3	7.9	8.4	5.4	7.2
6 HOUR																	
WT	n	5	4	8	7	6	6	6	3	5			3	6	6	6	3
	$\bar{x}$	3.9	6.8	6.0	3.7	6.6	6.7	6.1	6.7	2.5			3.3	7.9	8.5	5.4	7.0
MT	n	5	4	8	7	6	6	6	3	5			3	5	6	6	3
	$\bar{x}$	3.8	6.6	5.8	3.8	6.0	7.0	6.1	6.8	2.5			3.3	8.0	8.9	5.5	7.2
Combined	n	10	8	16	14	12	12	12	6	10			6	11	12	12	6
	$\bar{x}$	3.9	6.7	5.9	3.7	6.3	6.8	6.1	6.8	2.5			3.3	8.0	8.7	5.4	7.1

		DPG (µmol/gHb)								pH							
		15	17	18	19	20	21	22	23	15	17	18	19	20	21	22	23
0 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	6	6	6	5	3
	$\bar{x}$	9.6	22.9	15.3	9.5	18.8	22.1	20.8	21.7	7.34	7.62	7.59	7.54	7.56	7.57	7.54	7.42
MT	n	6	4	9	7	6	6	6	4	6	4	9	6	6	6	5	3
	$\bar{x}$	9.7	22.6	17.0	10.9	20.3	21.4	21.4	21.5	7.34	7.59	7.59	7.52	7.56	7.60	7.55	7.42
Combined	n	12	8	18	14	12	12	12	8	12	8	18	12	12	12	10	6
	$\bar{x}$	9.6	22.8	16.1	10.2	19.6	21.7	21.1	21.6	7.34	7.61	7.59	7.53	7.56	7.59	7.55	7.42
2 HOUR																	
WT	n	6	4	9	7	6	6	6	4	6	4	9	5	6	6	6	4
	$\bar{x}$	9.5	24.1	17.7	8.3	20.7	23.3	21.5	21.9	7.27	7.43	7.36	7.35	7.42	7.44	7.38	7.26
MT	n	6	4	9	7	6	6	6	4	6	4	9	5	6	6	6	4
	$\bar{x}$	9.7	24.6	18.6	7.6	21.4	23.5	22.3	21.6	7.28	7.41	7.37	7.36	7.42	7.46	7.39	7.27
Combined	n	12	8	18	14	12	12	12	8	12	8	18	10	12	12	12	8
	$\bar{x}$	9.6	24.3	18.2	8.0	21.0	23.4	21.9	21.7	7.27	7.42	7.37	7.35	7.42	7.45	7.38	7.27
4 HOUR																	
WT	n	6	4	8	7	6	6	6	4	6	4	8	6	6	5	6	4
	$\bar{x}$	8.0	23.7	17.3	5.8	21.5	23.2	21.8	18.6	7.18	7.32	7.24	7.27	7.32	7.35	7.29	7.20
MT	n	6	4	8	7	6	6	6	4	6	4	8	6	6	5	6	4
	$\bar{x}$	7.7	24.5	17.2	5.9	19.7	23.1	21.1	18.5	7.19	7.30	7.23	7.27	7.32	7.37	7.30	7.19
Combined	n	12	8	16	14	12	12	12	8	12	8	16	12	12	10	12	8
	$\bar{x}$	7.8	24.1	17.2	5.8	20.6	23.1	21.4	18.5	7.19	7.31	7.23	7.27	7.32	7.36	7.29	7.19
6 HOUR																	
WT	n	6	4	8	7	6	6	6	4	5	4	6	5	6	6	6	3
	$\bar{x}$	5.2	21.6	15.0	3.2	18.0	22.8	18.7	14.8	7.12	7.21	7.13	7.20	7.24	7.28	7.21	7.13
MT	n	6	4	8	7	6	6	6	4	4	4	5	5	6	6	6	3
	$\bar{x}$	4.4	21.4	12.8	2.4	20.8	23.2	19.0	17.3	7.12	7.19	7.12	7.19	7.24	7.32	7.22	7.13
Combined	n	12	8	16	14	12	12	12	8	9	8	11	10	12	12	12	6
	$\bar{x}$	4.8	21.5	13.9	2.8	19.4	23.0	18.9	16.1	7.12	7.20	7.13	7.19	7.24	7.30	7.22	7.13

APPENDIX C

Morphologic Characteristics of

Water Bath and Microwave Thawed Blood Samples

(Units Combined)

		%R (%)	%H (%)	Hb_t% (gm%)	Hb_S% (gm%)	Hct (%)	Hb_C% (gm%)	RBC# ($x10^6/mm^3$)	MCV (μ^3)	MCHC (%)	MCH ($\mu\mu g$)
POST THAW											
WT	n	51	51	51	51	51				51	
	$\bar{x}$	96.6	4.4	20.2	2.4	61.2				31.6	
MT	n	50	50	50	50	50				50	
	$\bar{x}$	97.3	3.8	20.4	2.1	61.3				32.0	
Combined	n	101	101	101	101	101				101	
	$\bar{x}$	96.9	4.1	20.3	2.2	61.3				31.8	
0 HOUR											
WT	n	48	48	48	48	48	48	47	47	48	47
	$\bar{x}$	80.3	0.9	10.7	0.1	28.0	10.6	3.6	78.1	38.2	29.8
MT	n	48	48	48	48	48	48	45	45	48	45
	$\bar{x}$	79.7	1.0	10.6	0.2	27.7	10.5	3.5	77.3	38.3	29.6
Combined	n	96	96	96	96	96	96	92	92	96	92
	$\bar{x}$	80.0	0.9	10.7	0.1	27.8	10.6	3.6	77.7	38.3	29.7
2 HOUR											
WT	n		48	48	48	48	48	42	42	48	42
	$\bar{x}$		1.1	10.8	0.2	28.1	10.7	3.6	76.8	38.0	29.5
MT	n		48	48	48	48	48	42	42	48	42
	$\bar{x}$		1.1	10.8	0.2	28.0	10.7	3.6	76.3	38.4	29.5
Combined	n		96	96	96	96	96	84	84	96	84
	$\bar{x}$		1.1	10.8	0.2	28.1	10.7	3.6	76.6	38.2	29.5
4 HOUR											
WT	n		47	47	47	47	47	41	41	47	41
	$\bar{x}$		1.2	10.8	0.2	28.3	10.7	3.6	77.3	37.9	29.5
WT	n		47	47	47	47	47	41	41	47	41
	$\bar{x}$		1.1	10.8	0.2	27.9	10.7	3.6	76.8	38.4	29.7
Combined	n		94	94	94	94	94	82	82	94	82
	$\bar{x}$		1.2	10.8	0.2	28.1	10.7	3.6	77.1	38.1	29.6
6 HOUR											
WT	n		47	47	47	47	47	41	41	47	41
	$\bar{x}$		1.5	10.9	0.2	28.3	10.7	3.7	76.7	38.0	29.4
MT	n		47	47	47	47	47	41	41	47	41
	$\bar{x}$		1.5	10.7	0.2	28.0	10.6	3.6	76.8	38.1	29.5
Combined	n		94	94	94	94	94	82	82	94	82
	$\bar{x}$		1.5	10.8	0.2	28.1	10.6	3.6	76.8	38.1	29.4

APPENDIX D

Metabolic Characteristics of

Water Bath and Microwave Thawed Blood Samples

(Units Combined)

		Glucose (μmol/gHb)	Glucose Rate of Decrease (μmol/gHb · min)	Lactate (μmol/gHb)	Lactate Rate of Increase (μmol/gHb · min)	Pyruvate (μmol/gHb)	ATP (μmol/gHb)	GSH (μmol/gHb)	DPG (μmol/gHb)	pH
0 HOUR										
WT	n	47	43	42	42	20	48	30	48	45
	$\bar{x}$	75.4	10.0	15.4	18.9	0.18	6.0	6.0	16.9	7.53
MT	n	48	47	42	42	20	47	30	48	45
	$\bar{x}$	77.5	10.1	17.0	18.3	0.18	6.0	6.1	17.6	7.53
Combined	n	95	90	84	84	40	95	60	96	90
	$\bar{x}$	76.5	10.0	16.2	18.6	0.18	6.0	6.0	17.2	7.53
2 HOUR										
WT	n	48	45	42	41	20	47	30	48	46
	$\bar{x}$	64.8	8.0	38.8	15.9	0.19	5.8	6.3	17.7	7.37
MT	n	48	41	42	41	20	47	30	48	46
	$\bar{x}$	65.3	8.2	38.3	15.9	0.20	5.8	6.2	18.1	7.37
Combined	n	96	86	84	82	40	94	60	96	92
	$\bar{x}$	65.1	8.1	38.5	15.9	0.20	5.8	6.2	17.9	7.37
4 HOUR										
WT	n	47	42	41	41	20	46	30	47	45
	$\bar{x}$	56.6	7.9	57.2	15.5	0.41	5.9	6.1	16.9	7.27
MT	n	47	46	41	40	20	46	30	47	45
	$\bar{x}$	58.8	7.2	57.8	14.3	0.44	5.7	6.0	16.6	7.27
Combined	n	94	88	82	81	40	92	60	94	90
	$\bar{x}$	57.7	7.5	57.5	14.9	0.43	5.8	6.0	16.7	7.27
6 HOUR										
WT	n	46	45	41	41	20	45	29	47	41
	$\bar{x}$	48.1	7.6	75.8	16.8	1.47	5.7	6.0	14.4	7.20
MT	n	46	46	41	41	20	45	28	47	39
	$\bar{x}$	50.1	7.8	74.4	15.9	1.48	5.6	6.1	14.4	7.20
Combined	n	92	91	82	82	40	90	57	94	80
	$\bar{x}$	49.1	7.7	75.1	16.4	1.48	5.7	6.0	14.4	7.20